GENETICS

A PERIODICAL RECORD OF INVESTIGATIONS
BEARING ON HEREDITY AND
VARIATION

VOLUME 14 - 1929

WITH NINETEEN PLATES AND ONE HUNDRED AND THIRTY-ONE TEXT FIGURES

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GENETICS

A PERIODICAL RECORD OF INVESTIGATIONS
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VARIATIONS

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CORRIGENDA

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Page 488, line 4, for "Russia" read "Union of Soviet Socialistic Republics."
Page 488, footnote 1, for "U. S. S. Russia" read "U. S. S. R."
Page 513, line 23, for "C," read "F," and for "i" read "j."
Page 513, line 3, for "G" read "AG."
Page 515, table 2, total under R, for "323" read "233".
Page 516, table 3, column 11, for "$(T_3)_4$" read "$(T_3)V_4$."
Page 518, table 4, column 7, for "m_6" read "m_1."
Page 518, table 4, column 12, for "m_{37}" read "m_3."
Page 520, line 1, for "(aa)" read "(bb)."
Page 520, line 29, add "deviation from 50 percent" after "exceeding each."
Page 520, line 32, for "*AM*" read "*CR*."
Page 520, line 33, add "the deviations from 50 percent" after "exceeding."
Page 522, table 6, column xy, row CR, for "49" read "39."
Page 525, line 21, for "v" read "u, v, w."
Page 525, line 22, for "D" read "H."
Page 527, table 11, heading, line 3, for "12" read "10."
Page 527, line 8, for "U" read "u, v, w."
Page 528, table 12, last column, for "-3.2" read "-2.3."
Page 543, last line, for "inpressed" read "impressed."

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Page 25, table 16, for "C_cx_c" read "X_cx_c."
Page 30, table 26, for "A_tB" read "A_tb"
Page 41, line 6, for "*Strapp*" read "*Stapp*."
Page 49, table 10, column 9, for "*Langsdorfii*" read "*Langsdorffii*."
Page 49, table 10, column 21, for "*Melomgena*" read "*Melongena*."
Page 53, line 7, "*Nelongena*" read "*Melongena*."
Page 53, legend for figure 3, for "*Melogena* read "*Melongena*."
Page 54, line 5, for "*rustical*" read "*rustica*."
Page 76, line 30, for "*Sarrdina*" read "*Sardiña*."
Page 76, line 42, for "Technik und Methodik des" read "Practische Anleitung zur Ausführung des."
Page 93, formula 10, for "$4R$" read "$4T$."
Page 97, line 5 from bottom, for "productivity" read "productively."
Page 103, last line of figures, for "$(T+S_1/2)^2$" read "$(T+S/2)^2$."
Page 107, table column headed F_2, for "$\mathcal{Z}/4$" read "2/4."

Page 120, lines 11 and 12, for "such equilibrium" read "such population to seek a new equilibrium."

Page 124, formula 20, for " -1 1/L" read "$1-1/L$."

Page 163, table 1 heading, for "**S**" read "S."

Page 324, figure 9a legend, for "paired" read " pairs of."

Page 425, line 5, for "$n+\frac{n_1 c}{2}=n$" read "$n_1+\frac{n_1 c}{2}=n$."

Page 430, line 5, for "4 genes in the parents" read "2 genes in the parent."

Page 430, line 9 from below, for "$\frac{1}{2}r+\frac{1}{2}r^2$" read "$\frac{1}{4}r+\frac{3}{4}r^2$."

Page 435, line 4, for "$0.07125(r-r^2)$" read "$0.07125(r-r^2)$ percent."

Page 438, line 6, for "$2P_3+P_4=\frac{2c^3+18c^2+12c-16}{(2+c)(n-1)}$" read

$$\text{"}2P_3+P_4=\frac{2c^3+14c^2+8c-16}{(2+c)(n-1)}\text{."}$$

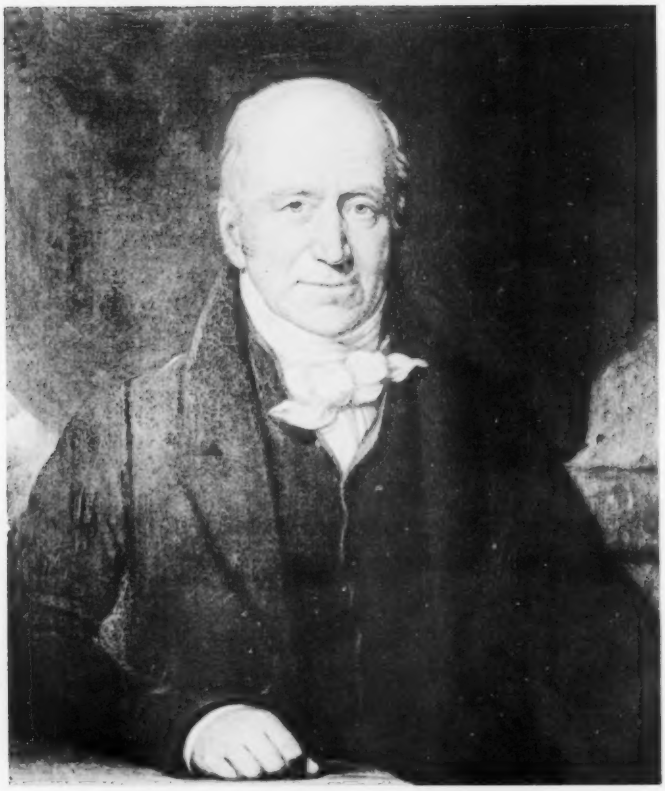
Page 438, line 9 from below, for "2104" read "1580."

Page 439, line 3, for "$2P_3+P_4=\frac{2c^2+6c-8}{n-1}$" read "$2P_3+P_4=\frac{2c^2+2c-4}{n-1}$."

Page 439, line 5, for "1053" read "703."

Page 501, table 9, last column, for "r. 18" read "4.18."

Page 514, line 9, for "$2p_1p_1P_2$ read "$2p_1p_1P_2p_2$."



THOMAS ANDREW KNIGHT

(FRONTISPIECE)

THOMAS ANDREW KNIGHT, F. R. S., L. S., distinguished plant-physiologist, horticulturist and plant and animal breeder, was born at Wormesley Grange near Hereford, England, August 12, 1759, and died at Downton Castle, Ludlow, England, on May 11, 1838. He was educated at Ludlow, and then in Doctor Crawford's school at Chiswick, later entering Baliol College, Oxford. He was painfully shy and was noted in college for his steady resistance to the intemperate habits so common in the colleges and universities at that time. He had a remarkable quickness of perception and of comprehension as well as a marvelous literary memory so that he excelled with little effort. Years later, he could repeat without error, whole pages of Virgil, Milton's *Paradise Lost*, and standard works of history and philosophy.

He married in 1791 Miss FRANCES FELTON and established himself at Elton, where he acquired a farm and a hothouse. His intellectual interest in problems of "natural history," horticulture, agriculture and animal husbandry was manifested in a long series of communications to the ROYAL SOCIETY of LONDON and the HORTICULTURAL SOCIETY OF LONDON which were published in the Philosophical Transactions and the Transactions of the HORTICULTURAL SOCIETY. He was particularly incited to place his observations in record by the distinguished botanist, Sir JOSEPH BANKS, and was also greatly encouraged by Sir HUMPHRY DAVY, the great chemist, with whom he carried on an intimate correspondence for many years. His first communication, read April 30, 1795, before the ROYAL SOCIETY, was "Upon the inheritance of decay among fruit-trees and the propagation of debility by grafting," and one of his last, published a few months before his death was "On the hereditary instinctive propensities of animals."

To combat the evident deterioration of many of the older horticultural varieties he urged the importance of developing new and better varieties by seedling selection following cross-breeding. His aims in breeding were strictly utilitarian rather than theoretical and he was the originator of many new varieties which came to have large significance for horticulture. A list of his more important new varieties includes:

Apples: Spring Grove Codling, Downton Lemon Pippin, Hereford Gilly-flower, Grange.

Pears: Monarch, Althorp Cressane, Rouse Level, Winter Cressane, Belmont.

Cherries: Elton, Waterloo, Black Eagle.

Plums: Ickworth Impératrice, Improved Damsons.

Nectarines: Impératrice, Ichworth, Downton, Althorp.

Strawberries: Elton, Downton.

Potatoes: Downton Yam.

Peas: Knight.

Cabbages: Improved varieties, unnamed.

In animal breeding he became known as an improver of the breed of Herefordshire cattle, and his cattle frequently took prizes at the animal shows in Herefordshire and at Smithfield. He hybridized Merino and Ryeland sheep in 1799 and followed the hybrid progenies for a number of years; and he also imported Norwegian ponies and mated them with the London Dray-Horse, producing hybrids with the good qualities of both parents.

He amused himself by tracing in man as well as in his experimental plants and animals, the inheritance of some traits from one parent, some from the other, "which he was disposed to imagine might be reduced to something like rule;"[1] but although clever in devising experiments, and philosophical in drawing conclusions and explaining the results of his experiments, he seems to have been not statistically inclined and most of his contributions are generalized statements of conclusions accompanied by a few illustrative examples, or from general impressions based on a wide range of unanalyzed experiments or experiences. His work with peas was certainly extensive enough to have given the whole Mendelian story, had he become interested in the statistical results, for he says: "I believe it would not be easy to suggest an experiment of cross-breeding upon this plant (the pea), of which I have not seen the result through many successive generations," and he describes a cross between a variety with gray seed-coats and one with transparent seed coats, in which the F_1 hybrids were backcrossed to the recessive type, giving exactly the Mendelian sequence of events but without noting the fact that the segregation gave a 1:1 ratio and necessitated a conclusion as to the constitution of the uniting gametes.

KNIGHT's more strictly scientific papers dealt with problems of plant physiology, such as the ascent and descent of sap, the nature of the cambium, the geotropism of roots and stems, the phototropism of tendrils, and so forth. He recognized the cauline nature of the hypocotyl, the maternal nature of seedcoats, and was also a pioneer in phytopathology.

In 1804 he assisted actively in founding the HORTICULTURAL SOCIETY OF LONDON, and he wrote the Introduction to the first volume of the Transactions of the Society when this publication was established in 1807. In 1810 he became the second President of the Society, which position he held continuously for 28 years, until his death in 1838. Under his guidance the Society started an experimental garden at Kensington in 1818, but four years later this was abandoned because of its close proximity to London and the resultant injury from the gas laden atmosphere of the city. In 1822 the present experimental garden of 33 acres was established at Wisley.

Besides holding membership in the ROYAL SOCIETY OF LONDON, the ROYAL SOCIETY OF EDINBURGH, the ROYAL BOTANICAL SOCIETY OF GLASGOW, and the HORTICULTURAL SOCIETY OF LONDON, THOMAS ANDREW KNIGHT was an honorary or corresponding member of scientific societies and academies in Germany (3), France, Sweden, Russia, Australia, Cuba, Canada, and the United States (7). He received eleven medals, including the ROYAL SOCIETY Gold Copley medal, the silver Banksian medal, the MASSACHUSETTS AGRICULTURAL SOCIETY medal, and the Grand Silver medal of the SWEDISH ACADEMY OF AGRICULTURE.

The portrait here reproduced was painted from life by SOLOMON COLE and is now in the possession of the ROYAL BOTANIC GARDENS, Kew, to whose Director we are greatly indebted for having had the reproduction made for us. The signature has been kindly supplied by C A. BOUGHTON-KNIGHT, Esq., a grand nephew of THOMAS ANDREW KNIGHT, and the present occupant of Downton Castle. Mr. BOUGHTON-KNIGHT also supplied much of the information from which the present sketch has been prepared.

[1] Quoted from the biography prepared by his family in 1840, and published by Longman. Orme, Brown, Green & Longmans, London, 1841.

LINKAGE STUDIES IN BARLEY[1]

D. W. ROBERTSON

Colorado Agricultural Experiment Station, Fort Collins, Colorado

Received May 1, 1928

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[1] A thesis presented to the faculty of the Graduate School of the UNIVERSITY OF MINNESOTA in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

The writer wishes to express his appreciation to Dr. H. K. HAYES, Professor of Plant Genetics, UNIVERSITY OF MINNESOTA, under whose direction these investigations were conducted, for his many valuable suggestions. The writer is also indebted to F. J. STEVENSON, Assistant Professor of Plant Genetics, UNIVERSITY OF MINNESOTA, for helpful criticisms of the manuscript.

Practically all of the data have been obtained at the COLORADO AGRICULTURAL EXPERIMENT STATION at Fort Collins. The writer wishes to gratefully acknowledge the cooperation of Director C. P. GILLETTE and Professor ALVIN KEZER in providing facilities for this investigation. The writer is also indebted to G. W. DEMING for assistance in collecting data.

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INTRODUCTION

The present conception of the chromosome theory of heredity is that the genes are arranged in a stable, orderly, linear manner in the chromosome; that genes in separate chromosome pairs are inherited independently and that there is the same number of groups of linked genetic factors as homologous pairs of chromosomes. The degree of linkage is in general considered to be correlated directly with the distance apart of the factors in the chromosome. *Drosophila melanogaster* has four pairs of chromosomes and some 400 factors have been located in them. Maize which has been studied more extensively than any other cereal crop has more than 35 factors located in eight of the ten pairs of chromosomes. With other cereal crops only a few isolated groups have been observed. There are a considerable number of easily distinguishable character pairs in cultivated barley and only 7 pairs of chromosomes. Barley can be grown satisfactorily under many different environmental conditions and for these reasons it appears desirable to use this organism in studies of linkage relations.

Arrangements have been made whereby the Minnesota, Colorado and Oklahoma stations are cooperating in studying linkage relations in barley. The present paper is a report of studies conducted in Colorado.

REVIEW OF LITERATURE

A fairly extensive review of the published papers on linkage relations in barley was made by GRIFFEE (1925) and only those papers which have a direct bearing on the present studies are reviewed.

Chlorophyll Deficiencies

An albino seedling is reported by WIEBE (1924) which was found in the F_3 head row of a cross between Nepal and a many noded dwarf. From later studies it was concluded that this Albino was due to a single

recessive factor. NILSSON-EHLE (1922) describes six chlorophyll mutations. Albino Nos. 1 and 3 occurred in six rowed barleys and Albino No. 2 in a two rowed variety. Yellow No. 1 was found in a six rowed barley and Yellow No. 2 in a two rowed form. Chlorina, a pale green chlorophyll dificient type, occurred in Gold, a two rowed barley. All of the whites are recessive to green and the progeny of the selfed heterozygous green plants segregated in a simple Mendelian ratio of 3 green seedlings to 1 white. A linkage was found between Albino 3 and Chlorina. All of the other chlorophyll deficiencies were found to be independently inherited.

HALLQVIST (1926) made crosses between the following chlorophyll deficiencies: Albino 1, Albino 2, Albino 4, Xantha 1, Xantha 2, Chlorina, Superchlorina, Virescent 1, Virescent 3, Lutescens 1, and the abnormalities Dwarf and Linearis. Forty-two out of 78 possible combinations were made. In crosses involving different albinos a ratio of green to albino of 9 to 7 was obtained and in a cross between a virescent and a xantha; green, xantha, and virescent seedlings occurred in a ratio of 9:3:4. In crosses in which Dwarf 1 was used a deficiency in the number of Dwarfs was found. This was explained by the fact that the Dwarf gametes in fertilization take part in fewer numbers than the normal gametes. In one combination the obtained ratio comes very close to the calculated with a deficit of 28 percent in the Dwarf gametes. The linkage of Albino 3 and Chlorina, found by NILSSON-EHLE, was confirmed and a linkage of Albino 4 with Albino 3 and Chlorina was found. The crossover percentages were found to be 10.2 for Albino 3 and Chlorina, 3.8 between Chlorina and Albino 4 and 12.5 between Albino 3 and Albino 4. The same author (1923) observed the effect of different temperatures on the development of chlorophyll in a yellowish white seedling. At a temperature of about 5°C no chlorophyll was developed. At a temperature between 12°–15°C, the seedlings produced chlorophyll in the tips of the leaf. After several days the chlorophyll deficient seedlings died. In temperatures of about 20°C, the lack of chlorophyll could not be detected in the young seedlings. The plants that developed past the seedling stage presented a somewhat stunted appearance. In some cases ears were formed but in many cases these were abnormal.

COLLINS (1927) describes an albino which can be differentiated at temperatures lower than 45°F. When grown at temperatures above 65°F, no albino plants develop. A single recessive gene is responsible for this chlorophyll deficient type. The F_2 segregation from the cross

six rowed albino × two rowed non-albino shows independent inheritance for the factor pairs concerned.

Inheritance of other characters

HAYES and GARBER (1927) summarize the literature on inheritance of the lateral florets as follows: "Two general results have been obtained from crossing two and six-rowed varieties. The most frequent result is an intermediate condition in the F_1 in which the lateral florets are awned, but produce little or no fruitfulness. In F_2 a 1:2:1 ratio of six-rowed, intermediate, and two-rowed forms are obtained. Six- and two-rowed forms breed true to these respective characters in later generations. Results of this nature can easily be explained on a single main factor difference. A cooperative study carried on at the MINNESOTA EXPERIMENT STATION has shown the probable origin of some *intermedium* forms. In a cross between Manchuria, a six-rowed barley, and Svanhals, a two-rowed variety, the F_1 was slightly fruitful and produced intermediate awns on the lateral florets. In F_2 a wide range of forms was obtained. The genetic nature of the F_2 plants was determined by growing seed of each in F_3. From the F_3 results it was possible to classify F_2 plants as follows:

1. Those that bred true for the six-rowed character.
2. Those that segregated, giving six-rowed, awned intermediate forms with very high fruitfulness of the lateral florets and *intermedium* forms in a 1:2:1 ratio.
3. *Intermedium* forms that bred true, giving few or no awns on the lateral florets and producing approximately 50 percent of barren lateral florets.
4. Those that gave all forms as in F_2.
5. Those that produced intermediates and two-rowed types.
6. Those that produced six-rowed, awned intermediates with little or no fruitfulness in the lateral florets and two-rowed forms in a 1:2:1 ratio.
7. Those that bred true for the two-rowed condition.

Results were explained by considering the Manchuria parent to contain two factors, one for six-rowed and one for *intermedium*, which was hypostatic to the six-rowed factor. It was thought possible that minor modifying factors were sometimes present which influenced the degree of fruitfulness of the lateral florets. Crosses between *intermedium* and six-rowed forms gave intermediates of high fruitfulness in F_1 and a ratio in F_2 which indicate a single factor difference. *Intermedium* forms crossed

with two-rowed, gave awnless forms with very low fruitfulness in F_1 and a ratio indicating one main factor difference in F_2.

In a cross of *Hordeum deficiens steudelii* × Manchuria, GRIFFEE (1925) reports a single factor difference for six-rowed and *deficiens*. In another cross between Svanhals and Lion two main factor pairs were found to be responsible for fertility of the lateral florets. The conclusion was reached that the *intermedium* factor may be carried by a six-rowed variety and that the presence of this factor does not modify the expression of the factor for the six-rowed condition. Two-rowed lacks the factors for intermedium and six-rowed. Apparently the factor pairs which differentiate six-rowed *versus* two-rowed and *intermedium versus* two-rowed are independently inherited.

In a contribution to the genetics and the morphology of barley, VEIDEMAN (1927) states: "The cause of sterility or fertility of the lateral spikelets of barley lies not in the presence or absence of reproductive organs in the lateral florets, as commonly thought but in the structure of the pedicel of the lateral florets, typical for each variety. The longer and narrower the pedicel, the feebler is the development of the lateral spikelet, and the reverse. Thus the fertility is not an independent hereditary character but the physiological results of the anatomical structure and the function of the pedicel. To put it differently: there exists no "gene for fertility."

HOR (1924) concludes that deficiens, distichon and vulgare are multiple allelomorphs. TEDIN and TEDIN (1926) in discussing the type of spike in a cross between a six rowed and a two rowed barley, suggest that the lateral florets with rounded tips be classified as two rowed. Such two rowed plants may produce somewhat inflated lateral florets, but these never bear kernels. Such types breed true for the two rowed condition. When the palea was more or less pointed the plant was classified as heterozygous and has always proved to be heterozygous by the breeding test. In F_3 there are three distinct groups of families, namely, constant six-rowed or two-rowed and families segregating for all three types; no families segregating in typical two-rowed and *intermedium* were observed. Their *intermedium* is similar to the intermediate of HAYES and GARBER (1927).

HAYES and GARBER (1927) list the following characters as being dependent upon single factor differences: Hooded *versus* awned, rough *versus* smoth awn, black palea *versus* colorless, purple palea *versus* colorless, hulled *versus* naked, and black pericarp *versus* white pericarp.

GRIFFEE (1925) found that the characters early heading *versus* late heading and intermediate smooth *versus* smooth awn depend upon single factor differences. HOR (1924) decided that long haired rachilla and short haired rachilla differ by a single genetic factor.

Linkage relations

Several linkage studies have been reported recently in barley and various conflicting results obtained. HAYES and GARBER (1927) give a rather extensive summary of the published data. GRIFFEE (1925) found that resistance and susceptibility to *Helminthosporium sativum* are definitely inherited. By correlating the reaction of F_3 lines to this pathogen in relation to other characters in F_2 the inerfence is drawn that at least three factors or groups of factors are concerned in the production of resistance of the type possessed by Svanhals. One factor or group of factors for reaction to *Helminthosporium sativum* was linked with the factor for two rowed, one with the factor for rough awn, and one with the factor for white glumes.

The factor for early heading was found to be linked with the factor for six-rowed. The linkage intensity was very low, the crossover value being 42 percent. The linkage for susceptibility to *Helminthosporium sativum* with the factor for earliness is much more intense than that with the factor for six-rowed or else earliness in itself predisposes the plant to attacks by the pathogen. From the results of the studies, four linkage groups have been established. HOR (1924) found that the long and short haired rachilla factors are independent of the allelomorphic series for fertility of the lateral florets and hairiness of the rachilla and hulled *versus* hullless were inherited independently of each other. A linkage is shown between factors for hairy glume and naked and hulled seeds. The percentage of crossing over is calculated as 25.32 ± 1.73. He also concludes that the factors for black versus white glume, rough versus smooth awn, and long versus short haired rachilla are in one group. Too small a number of individuals were studied in each case to be certain of his conclusions. NEATBY (1926) concludes from studies made on quantitative characters that maturity, height and density are not very closely linked with the factor pair which differentiates two- *versus* six-rowed. TEDIN and TEDIN (1926) found a correlation between the height of plant and the two-rowed type. The two-rowed segregates from a cross between six-rowed and two-rowed were, on the average, 10 cm taller than the six-rowed type. They conclude that this correlation most likely is due to linkage between the factor for two rowedness and a plant height factor.

In a more recent paper (1927) the same authors, in a study of development of the kernel basis and its relation to density, referred to three types of bases: vernum, with a transversal nick; falsum, without a nick, but with the dorsal side bevelled; spurium, without both the nick and the bevelling. The lax-eared nutens as well as the corresponding variety with fertile lateral florets, vulgare, always belong to the falsum type. The dense-eared erectum, as well as the extremely dense-eared zeocriton and the correspondingly dense-eared six-rowed variety hexastichum were all either vernum or spurium. They found that two complementary factors E_{r1} and E_{r2} are necessary for the development of the falsum-basis whereas the vernum basis may develop from $E_{r_1}E_{r_1}e_{r_2}e_{r_2}, e_{r_1}e_{r_1}E_{r_2}E_{r_2}$ or $e_{r_1}e_{r_1}e_{r_2}e_{r_2}$. They say in conclusion "In spite of the fact that conclusive proof cannot be given, we consider it highly probable, that E_{r_1} and E_{r_2} are linked with one another, with a crossing over between 33 and 40 percent."

MATERIAL AND METHODS

This paper presents a study of the inheritance of simple Mendelian factor pairs and their possible linkage relations with particular attention to the linkage of certain chlorophyll deficiencies. The following varieties have been used in crosses: Colsess, Trebi, *H. distichon nigrinudum*, Minnesota Selection 90–5, Minnesota Selection 90–8, and *H. deficiens nudideficiens*.

Description of parents

Colsess

This is a six-rowed, hooded barley with a hulled grain of a bluish green color. The straw and glume color is light yellow. The shank of the hood is about 5 mm long and is barbed at the base. The rachis is rather tough and the head does not shatter easily. The rachilla hairs are short and the outer glume is covered with very short hairs.

Trebi

Trebi is described by HARLAN, MARTINI, and POPE (1925) as a pure line selection made in 1907 in the cooperative breeding experiments conducted by the U. S. DEPARTMENT OF AGRICULTURE and the MINNESOTA AGRICULTURAL EXPERIMENT STATION at St. Paul, Minn. It is a six-rowed, bearded, hulled barley with heads very similar to those of Coast. Under Colorado conditions (irrigated) the kernels are large and bluish in color. The straw is weak. In threshing the awns break off from the glume more easily than those of the Coast variety.

Hordeum distichon nigrinudum (HARLAN 1918)

This is an awned, two-rowed barley, with a hullless black grain and black glumes. The naked seeds have a charred appearance. The straw color is purple which is most marked in the sheath of the leaves of the upper node. The rachilla hairs are short.

Minnesota Selection 90–5

This is a two-rowed barley of the distichon type. It has black, smooth awns. The grain and glumes are black, the rachilla hairs are long and the central rib of the outer glume has long fine hairs.

Minnesota Selection 90–8

This is similar to Minnesota Selection 90–5 with the exception that the head is a little more lax.

H. deficiens nudideficiens (HARLAN 1918)

This variety is a rough awned, two-rowed barley with a hullless grain. The grain is colorless, the rachilla hairs are long and the rachis is tough. Under Colorado (irrigated) conditions this barley is very early.

Methods of conducting experiments

In this study of the various differential characters particular attention has been given to certain chlorophyll deficiencies. As plants which are homozygous recessive for the chlorophyll defective seedling factors cannot be grown to maturity, plants heterozygous for genetic factors for green and white seedlings were used as one or both of the parents. When one parent was heterozygous for green and white seedlings, two types of F_1 plants were obtained; one being homozygous for the green seedling factors and the other heterozygous for the green and white seedling factors. In crosses in which both parents were heterozygous for different chlorophyll deficiencies four types of F_1 plants were obtained.

By testing seedling progeny of F_1 plants in the greenhouse, it was possible to determine fairly accurately their genetic nature for chlorophyll deficiencies. Particular genetic types were used to make more extensive studies in the later generations.

Studies of inheritance of botanical character differences were made for the most part in crosses where chlorophyll deficiencies were not involved.

INHERITANCE OF SIMPLE MENDELIAN CHARACTERS

White seedlings in Colsess

Head selections were made in the F_{11} generation of Colsess barley in 1922. The progeny of several of the selections were noticed to contain white seedlings. These seedlings died in about a week or ten days after germination. Counts of white seedlings were made in the F_{12} generation in three strains which were later numbered, Colsess I, Colsess II, and Colsess III. The observed ratios of green to white seedlings in the F_{12} generation were very close to a 3 to 1 ratio. The deviation divided by the probable error[1] was 1.7 in the case of Colsess I; 0.98 in the case of Colsess II, and 2.6 in the case of Colsess III. Progeny from seed of these plants were tested for white seedlings in the greenhouse in F_{13}. From these studies the following conclusions were drawn. Colsess No. III agrees very well with a 3 to 1 ratio of green to white seedlings on the basis of data from the F_{12} and F_{13} generation. The segregation obtained from Colsess I fits closely to a 3 to 1 ratio of green to white seedlings in the F_{12} and F_{13} generation. Colsess No. II, while indicating a 3 to 1 ratio of green to white seedlings, later developed a strain which approaches more closely to a 9 to 7 ratio. This latter strain was not used in the linkage studies.

In the F_1 generation of crosses between Colsess I and Colsess III, white seedlings appeared. This indicates that these two lines are dependent upon the same genetic factors for white seedlings. In crosses between Colsess I and Colsess II and between Colsess II and Colsess III similar results were obtained. The number of homozygous and heterozygous F_2 lines from crosses where both parents were heterozygous for white seedlings is given in table 1. In all cases where the parent plants used in the crosses were heterozygous for white seedling factors both green and white seedlings were obtained in the F_1. This again indicates that the white seedlings in Colsess I, II, and III are the result of the reaction of the same factor pair. This is further borne out by studying the progeny of heterozygous F_1 plants.

With the exception of crosses No. 58 and 59 the fits are good to the expected 3 to 1 ratio. The number of white seedlings in cross No. 59 is low. In cross 58D, divided by P.E. was 10.86 and the number of white seedlings was much larger than expected on a 3 to 1 basis. In conclusion it can be stated that the white seedlings in all crosses involving Colsess I,

[1] Tables of probable errors of Mendelian ratios prepared by the Department of Plant Breeding of CORNELL UNIVERSITY, Ithaca, New York, were used to determine the probable errors.

II, and III, except cross No. 58, are due to the reaction of the same factor pair.

TABLE 1

Seedling counts in the F_1 generation of Colsess×Colsess crosses and the number of segregating and pure green families in the F_1.

CROSS	CROSS NUMBER	F_1 SEGREGATION		NUMBER OF F_2 SEGREGATING FAMILIES	NUMBER OF F_2 PURE GREEN FAMILIES
		Green	White		
Colsess I ×Col. II	12	8	8	5	3
Colses I ×Col. II	59	13	2	7	4
Colses I ×Col. III	92	11	3	5	6
Colses I ×Col. III	91	10	6	7	3
Colses I ×Col. III	15	11	4	7	4
Colsess III ×Col. II	350	10	6	6	4
Colsess III ×Col. II	352	10	5	7	4
Colsess II ×Col. III	50	12	4	6	6
Colsess II ×Col. III	58	10	5	8	3
Observed Total.....		95	43	58	37
Calculated Total.....		*103.5*	*34.5*	*63.34*	*31.67*

A cooperative agreement has been reached by several workers to accept *aa* which was first used by NILSSON-EHLE to denote the genotypes of white seedlings in barley. NILSSON-EHLE subscribes his letters with the numbers 1, 2, etc., to denote genetically different whites. Until such times

TABLE 2

Segregation in F_2 for green and white seedlings in crosses between the different Colsess strains.

CROSSES	CROSS NUMBER	OBSERVED RATIO		DEVIATION IN NUMBERS	$\frac{D}{PE}$
		Green	White		
Colsess I ×Col. II	12	80	26	0.5	0.17
Colsess I ×Col. II	59	1936	584	46.0	3.14
Colsess I ×Col. III	92	143	39	6.5	1.65
Colsess I ×Col. III	91	223	67	5.5	1.11
Colsess I ×Col. III	15	1257	384	26.25	2.22
Colsess III ×Col. II	350	94	37	4.25	1.27
Colsess III ×Col. II	352	171	57	0	0
Colsess II ×Col.III	50	801	272	3.8	0.40
Colsess II ×Col. III	58	2012	898	170.5	10.85

as the white seedlings are tested with NILSSON-EHLE's material, a letter representing the variety in which the particular whites first occurred will be used as a subscript to denote a particular white seedling. If more than one white occurs in a variety, a subscript to the letter will be used until

it is identified with a white in some other variety. Thus the factor pair for the white seedling condition in the Colsess strains is designated as $A_c a_c$.

Cross No. 58 which deviated widely from a 3:1 ratio of green to white was studied in later generations. The different segregations of the F_2 plants from this cross are presented in table 3.

TABLE 3

The observed segregation in F_2 compared with calculated 3:1 and 9:7 ratios of white and green seedlings in Cross No. 58.

FAMILY NUMBER	NUMBER SEEDLINGS	3:1 RATIO			DEVIATION IN NUMBERS	D/PE
			Green	White		
1	104	obs.	80	24		
		cal.	*78*	*26*	2	0.67
2	272	obs.	200	72		
3		*cal.*	*204*	*68*	4	0.83
3	344	obs.	183	161		
		cal.	*258*	*86*	75.0	13.84
4	495	obs.	380	115		
		cal.	*371.25*	*123.75*	8.75	1.35
6	1106	obs.	843	263		
		cal.	*829.5*	*276.5*	13.5	1.38
7	87	obs.	60	27		
		cal.	*65.25*	*21.75*	5.25	1.93
10	502	obs.	266	236		
		cal.	*376.5*	*125.5*	110.5	16.90
		9:7 ratio				
3	344	obs.	183	161		
		cal.	*193.5*	*150.5*	10.5	1.69
10	502	obs.	266	236		
		cal.	*282.38*	*219.62*	16.38	2.18

Families No. 5 and 8 were all pure green.

Five families out of nine segregated in a 3 to 1 ratio, two segregated in a 9 to 7 ratio and two were homozygous for green. This type of segregation would be obtained if one of the parents was heterozygous for two complementary factor pairs for green versus white seedlings and the other parent carried only one of these factor pairs in the heterozygous condition. In the F_1 a 3:1 ratio of green and white seedlings would be expected. In F_2 a ratio of 1 homozygous green to 3 segregating in a 3:1 ratio and two in a 9:7 ratio would be expected. The obtained ratios fit

the expected very well. The segregation of the immediate progeny of the parents of cross No. 58 are of interest although the numbers are small. Colsess II gave 21 green to 17 white seedlings and Colsess III 29 green to 10 white seedlings. Colsess II fits well to a 9 to 7 ratio while Colsess III fits a 3:1 ratio.

FIGURE 1.—White Seedlings in Colsess (right) and Trebi (left).

It is apparent that this strain of Colsess II carries two complementary factors for chlorophyll color. The strain will be studied more extensively for the purpose of isolating and obtaining linkage relations with the second white seedling factor.

White seedlings in Trebi

These were observed originally at the MINNESOTA STATION. A plant of this strain was grown in Colorado in 1924. Progenies of this plant were later grown in 1925, 1926, and 1927. As all the Trebi plants producing white seedlings are descendants of this single individual, the counts of seedlings made in the greenhouse and field from 1925 to 1927 are summarized together. In all, 829 seedlings have been counted, from parent plants heterozygous for green and white seedling factors; of these 616 were green and 213 were white. The expected ratio on a three to one basis for the above number of individuals is 621.75 green to 207.25 white. The obtained ratio deviates 5.75 from the expected. The deviation divided by the probable error (D/PE) is less than one which indicates that a single factor pair is involved. Phenotypically the white seedlings in Colsess and in Trebi I cannot be told apart (see figure 1).

Xantha seedlings in Colsess

This refers to yellow seedlings originally observed in a selection from Colsess, named Colsess IV. In the field the color is picric yellow (RIDGWAY, Plate IV). In the greenhouse the color is classified as Bright Chalcedony yellow by the use of RIDGWAY's tables (Plate XVII). This chlorophyll deficiency is modified in appearance by differences in temperature. As controlled conditions were not available, the definite thermal point for the development of chlorophyll was not learned. One plant of the xantha type was grown to maturity and the line continued for three generations. Seedling counts, of the progeny of parent plants which were heterozygous, were made both in the field and in the greenhouse. The following results were obtained:

TABLE 4

Segregation for green versus xantha in progeny of Colsess IV.

GREEN	XANTHA	DEVIATION IN NUMBERS	$\frac{D}{PE}$
1004	375	30.25	2.80

A single factor difference is responsible for the xantha seedling in Colsess IV.

Black versus white glume color

Studies were made in the following crosses: Colsess×*H. distichon nigrinudum*, Colsess×Minnesota 90–5, Colsess×Minnesota 90–8, and

Trebi×Minnesota 90–5. The results indicate that black *versus* white glume color are dependent upon a single factor pair. The following table gives the F_2 segregation of black and white glumed plants in the above crosses.

TABLE 5

Segregation for black versus white glumes in the F_2 of crosses between black and white glumed parents.

CROSS	F_2 DATA		DEVIATION IN NUMBERS	D / PE
	Black	White		
Colsess×H. dis. nig.	3531	1192	11.25	0.56
Colsess×Minn. 90–5	785	255	5.00	0.53
Colsess×Minn. 90–8	224	88	10.00	1.94
Trebi×Minn. 90–5	674	187	28.25	3.30

Hoods versus awns

Studies were made in crosses between Colsess×*H. distichon nigrinudum*, Colsess×*H. deficiens nudideficiens*, Colsess×Minnesota 90–5, and Colsess ×Minnesota 90–8. The F_2 segregations approach a 3 to 1 ratio, indicating that a single factor pair is responsible for the hooded and awned condition. Table 6 gives the F_2 segregation of hooded and awned plants.

TABLE 6

Segregation of hooded versus awned plants in the F_2 of crosses between hooded and awned parents.

CROSS	F_2 DATA		DEVIATION IN NUMBERS	D / PE
	Hoods	Awns		
Colsess×H. dis. nigrinudum	3471	1252	71.25	3.55
Colsess×H. def. nudideficiens	2650	987	77.75	4.38
Colsess×Minnesota 90–5	797	243	17.00	1.80
Colsess×Minnesota 90–8	229	83	5.00	0.97

Covered versus naked seeds

The covered versus naked seed character pair was studied in crosses between Colsess×*H. distichon nigrinudum* and Colsess×*H. deficiens nudideficiens*. Table 7 gives the F_2 segregation of plants from the above crosses for the covered seed versus naked seed character pair.

From the following table it appears that the covered versus naked condition of the seed is dependent upon a single factor pair difference.

TABLE 7

Segregation of covered versus naked seed in the F_2 generation of crosses between Colsess×H. deficiens nudideficiens and Colsess×H. distichon nigrinudum.

CROSS	F_2 DATA		DEVIATION IN NUMBERS	D / PE
	Covered	Naked		
Colsess×H. dis. nigrinudum	3593	1130	50.75	2.53
Colsess×H. def. nudideficiens	2706	931	21.75	1.23

Long versus short haired rachilla

Studies were made in crosses between Colsess×*H. deficiens nudideficiens*, Colsess×Minnesota 90–5, Colsess×Minnesota 90–8, and Trebi ×Minnesota 90–5. The segregation of the F_2 generation shows that long versus short haired rachillas differ by a single factor pair. The F_2 data are presented in table 8.

TABLE 8

Segregation of long versus short haired rachilla in the F_2 generation.

CROSS	F_2 DATA		DEVIATION IN NUMBERS	D / PE
	Long	Short		
Colsess×H. def. nud.	2736	901	8.25	0.47
Colsess×Minn. 90–5	793	247	13.00	1.38
Colsess×Minn. 90–8	238	74	4.00	0.78
Trebi×Minn. 90–5	667	194	21.25	2.48

INHERITANCE OF FERTILITY OF THE LATERAL FLORETS

Studies were made in a cross between Colsess and *H. deficiens nudideficiens*. The F_1 generation was intermediate between six- and two-rowed. (Figure 2. No. 2.) The F_2 generation could not be classified accurately for fertility of the lateral florets. The progeny of the F_2 plants were grown in the F_3, and the F_2 phenotypes were determined from the F_3 segregation. Seven groups were established which is a similar condition to that described by HARLAN and HAYES (1920). As six-row, two-row and deficiens have been found to be allelomorphic no attempt has been made in these studies to distinguish between two row and deficiens. The results establish the genotype of the six-rowed parent as consisting of two factor pairs for fertility of the lateral florets. Colsess is therefore represented as *vv II*. Factor *v* is similar to the *A* factor and *I* to the *B* factor of Harlan and Hayes. Similar segregations were found for the following crosses, Colsess×Minnesota 90–5 and Trebi×Minnesota 90–5. Table 9 presents

the observed and the calculated segregation on the genetic hypothesis outlined. While there is a slight deviation, there is in general good agreement between the observed results and the calculated.

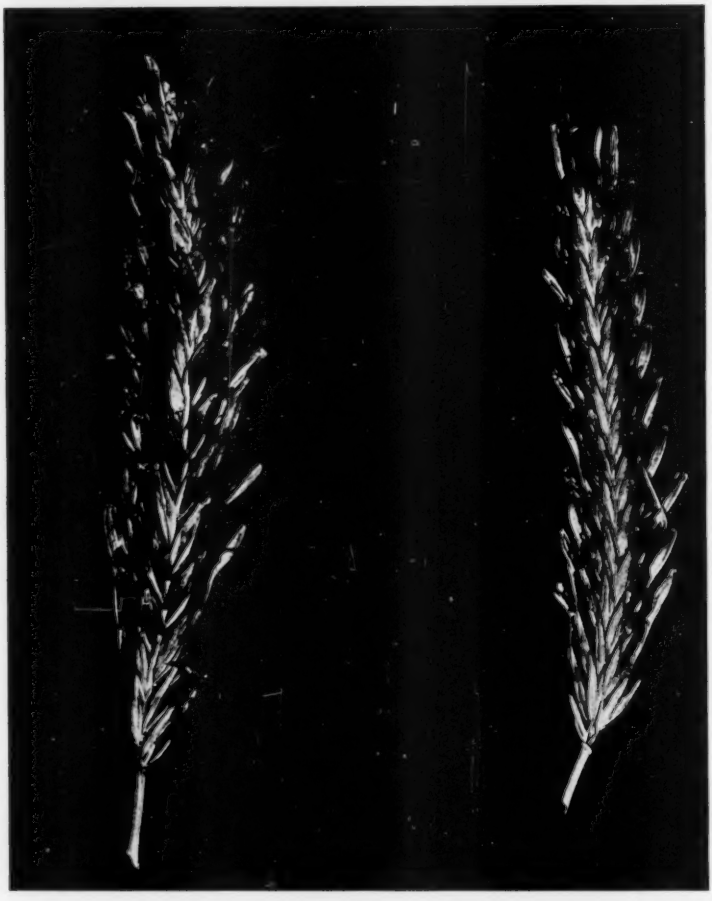

FIGURE 2.—Types of F_1 heads obtained from crosses between Colsess×*H. distichon nigrinudum* (left) and Colsess×*H. deficiens nudideficiens* (right).

In a cross between Colsess and *H. distichon nigrinudum* the F_1 plants were intermediate, figure 2, No. 1. The F_2 plants were classified as six-rowed and non-six-rowed. The six-rowed class includes all F_2 plants

TABLE 9

Segregation in F_2, as determined by F_3 breeding behavior, for fertility of the lateral florets.

F_2 GENOTYPE	CROSSES					
	Colsess×H. def. nud. observed	*calculated*	Colsess×Minnesota 90-5 observed	*calculated*	Trebi×Minnesota 90-5 observed	*calculated*
vvII	83	*88.7*	148	*123.3*	110	*119.2*
vvIi						
vvii						
vVIi	99	*88.7*	121	*123.3*	113	*119.2*
vVii	50	*44.4*	48	*61.6*	65	*59.6*
VVII	19	*22.2*	27	*30.8*	34	*29.8*
VVIi	39	*44.4*	48	*61.6*	61	*59.6*
VvII	45	*44.4*	73	*61.6*	58	*59.6*
VVii	20	*22.2*	28	*30.8*	36	*29.8*
Total	355	*355*	493	*493*	477	*476.9*
X^2		*3.51*		*13.82*		*3.47*
P		*.7414*		*.0320*		*.7487*

which produced only six rowed progeny in the F_3. Table 10 gives the F_2 segregation for non-six-rowed versus six-rowed.

TABLE 10

Segregation for non-six-rowed and six-rowed in the F_2 generation of a cross between Colsess and H. distichon nigrinudum.

SIX-ROWED	NON-SIX-ROWED	DEVIATION IN NUMBERS	$\frac{D}{PE}$
125	412	9.25	1.37

The segregation observed can be explained on the basis of single factor pairs for six-rowed versus non-six-rowed. No attempt was made to study the differences in the non-six-rowed group. Since the Colsess parent has been shown by previous crosses to contain the factor pair *vv* for six-rowed; the distichon parent has the gametic constitution *VV*.

INTERRELATIONS OF SIMPLE MENDELIAN CHARACTER PAIRS

Considerable information is already available regarding interrelations of genetic character pairs for the major botanical character differences. Consequently the interrelations for these will be presented briefly before giving the results of a study of the linkage relations of factor pairs for chlorophyll deficiencies with factor pairs for other character differences.

TABLE 11

Pure green families and segregating families summarized on the basis of closeness of agreement with 9:7 or 3:1 ratios.

		3:1 RATIO						9:7 RATIO			
		Observed		Calculated		Deviation in numbers	D / PE	Calculated		Deviation in numbers	D / PE
		Green	White	*Green*	*White*			*Green*	*White*		
Trebi×Colsess III											
II-25-3	1	516	164	*510*	*170*	6	0.79				
	2	465	117	*436.5*	*145.5*	28.5	4.04				
	3	421	119	*405*	*135*	16.	2.36				
	5	464	161	*468*	*156*	5.	.68				
	4	355	249	*453*	*151*	98	13.65	*339.75*	*264.25*	15.25	1.86
	8	533	335	*651*	*217*	118	13.72	*488.25*	*379.75*	44.75	4.54
	9	414	282	*522*	*174*	108	14.00	*391.5*	*304.5*	22.5	2.55
	6	All	Green								
	7	All	Green								
Trebi×Colsess III											
II-25-2	1	84	29	*84.75*	*28.25*	0.75	.23				
	2	All	Green								
Colsess I×Trebi											
II-25-50	1	96	21	*87.75*	*29.25*	8.25	2.61				
	5	106	25	*98.25*	*32.75*	7.75	2.32				
	6	34	20	*40.5*	*13.5*	6.5	3.02				
	11	177	48	*168.75*	*56.25*	8.25	1.88				
	2	40	32	*54.0*	*18.0*	14.0	5.65	*40.5*	*31.5*	0.5	0.02
	4	96	57	*114.0*	*38.0*	19.0	5.26	*85.5*	*66.5*	9.5	2.30
	12	159	106	*198.75*	*66.25*	*39.75*	8.16	*149.0*	*116.0*	10.0	1.83
	13	146	87	*174.75*	*58.25*	28.75	6.45	*131.0*	*102.0*	15.0	2.94
	3	All	Green								
	10	All	Green								
	14	All	Green								

INTERRELATIONS OF CHLOROPHYLL DEFICIENCIES

White seedlings in Colsess and Trebi

Crosses were made between Trebi which was segregating for green and white seedling A_tA_t and the heterozygous Colsess line called A_cA_c. Only those crosses were considered where the parent plants by progeny tests were known to be heterozygous for factor pairs for green and white seedlings. Three separate crosses of this kind were available. The number of F_1 plants was 9, 2, and 11, respectively, and all were green, which indicates that the factor pair in Trebi for green and white seedlings is different from the A_cA_c factor pair of Colsess. If the factor pairs are independently inherited, the calculated breeding behavior in F_2 is 1 pure green line, 2 heterozygous giving a ratio of 3 green:1 white and 1 segregating in a 9:7 ratio. The detailed results are given in table 11.

Of the 22 lines 6 were homozygous green, 9 agreed fairly well with a 3:1 ratio and 7 with a 9:7 ratio. In the 3:1 segregating lines there was a tendency for too few white seedlings. If there was linkage of the factor pairs for green versus white seedlings in Colsess and Trebi, it could be studied only in the lines where both factor pairs are involved. There is a consistent tendency for too few white seedlings in these lines, for linkage in the repulsion phase. The breeding behavior of the 22 F_2 lines is summarized in table 12. The agreement between observed and calculated for independent inheritance is good.

TABLE 12

The number of lines segregating in a 9:7 or a 3:1 ratio of green to white seedlings or producing only pure green seedlings.

	SEGREGATING LINES		PURE GREEN LINES
	9:7 ratio	3:1 ratio	
Number of lines	7	9	6
Calculated	*5.5*	*11*	*5.5*

It may be concluded, therefore, that the white seedlings in Trebi I are caused by the reaction of a different factor pair than A_cA_c which is responsible for the white seedlings in Colsess. The factor pair for green and white seedlings of Trebi I will be represented as A_tA_t.

White seedlings and xantha seedlings in Colsess

Crosses were made between lines which were heterozygous for the factor pairs A_cA_c green versus white and X_cX_c green versus xantha seedling.

Only those crosses were considered where both parent plants used proved to be heterozygous for the chlorophyll deficiencies which were being studied. In accordance with the factorial method used, $A_cA_cX_cX_c$ was crossed with $A_cA_cX_cx_c$. The F_1 seedlings were all green indicating that the factors causing white and xantha seedlings in Colsess are not allelomorphic. The F_1 plants should be of four different genotypes $A_cA_cX_cX_c$ producing only green seedlings in the F_2; $A_cA_cX_cx_c$ producing green and xantha seedlings in the proportion of 3:1; $A_cA_cX_cX_c$ producing seedlings in the ratio of 3 green to 1 white and $A_cA_cX_cx_c$ producing all three types of seedlings green, xantha, and white.

Forty-nine F_1 plants from crosses where both parents were heterozygous were obtained. The segregation for chlorophyll deficiencies was studied in F_2. On the basis of the breeding behavior in F_2, 13 F_1 plants proved homozygous for factors for green seedlings, 12 gave F_2 progeny segregating for green and xantha, 13 gave F_2 progeny segregating for green and white and 13 proved to be heterozygous for both the green versus xantha and green versus white factor pairs. This latter group gave an opportunity to study linkage relations of the two types of chlorophyll deficiencies. Because of the relatively few plants in the F_2 progenies it was necessary to make the study in F_3. The study was made by growing the progenies of F_2 green plants from F_2 lines segregating for green, xantha and white seedlings.

The calculated segregation on the basis of independent inheritance of the factor pairs concerned is as follows: One plant out of every 9 should be homozygous for both dominant factor pairs and should breed true for green seedlings. Four plants would be heterozygous for 1 factor pair and homozygous for the other factor pair. Green and xantha seedlings would be produced by the progeny of two of these plants and green and white seedlings would be produced by the progeny of the other two. The four remaining plants would be heterozygous for both factors and would give progeny consisting of green, xantha, and white seedlings. There were 47 F_2 plants which matured in lines which in F_2 segregated for green, xantha and white seedlings. The genetic nature of these was determined by their F_3 breeding behavior. The calculated ratio for independent inheritance and for the four classes of plants described above is 5.22:10.44:10.44:20.88. If the factor pair X_cx_c is inherited independently of the factor pair A_ca_c only 21 of the 47 plants tested should give green, xantha and white seedlings.

The progeny of all 47 plants, however, gave green, xantha and white

seedlings. This indicates a possible linkage of factors for xantha (x_c) and white (a_c). An attempt was made to determine the crossover value from the F_3 seedling counts from the 47 F_2 plants.

All of the F_3 segregations were in the repulsion phase so the F_3 seedling counts of the 47 F_2 plants were summarized.

A short method has been derived to determine the expected number of lines which would give the coupling type of linkage in the F_3 generation if the repulsion type was obtained in the F_2 generation. By the use of Emerson's gametic ratio represented by $r:s$ these calculations can be made. In the case of repulsion s would be higher than r. By squaring r and s the expected number of F_3 lines showing repulsion and coupling would be obtained. The same method can be used to determine the number of lines which will show repulsion in the F_3 when coupling is obtained in the F_2 generation. An example is given below with 10 percent crossing over and repulsion: $r=1$, $s=9$; therefore 1 line would segregate in the coupling phase to 81 lines segregating in the repulsion phase.

Table 13 gives the summary of the segregations of the F_3 progeny of 47 green F_2 plants from the families heterozygous for both factors in the F_2.

TABLE 13

The F_3 seedling counts of 47 green plants from the F_2 families heterozygous for both xantha and white seedlings from crosses between Colsess and Colsess IV.

CROSS	NO. OF F_2 GREEN PLANTS	SEEDLING RATIO				
		Green	Xantha	White	Total	X^2
Colsess × Colsess IV	35	8314	4120	3995	16531	
Colsess IV × Colsess	12	2869	1379	1460	5773	
Total	47	11183	5499	5455		
Observed ratio		11183	5499	5455	22304	
Calculated ratio		*12546*	*4182*	*5576*	*22304*	*565.4*

By examining the data in table 13, it will be seen that the xantha class has a larger number of individuals than the white class. This brings up the question: where do the double recessives belong? In a cross in which the same factor pair X_cx_c was studied in relation to green versus white seedlings in Trebi A_ia_i the white class undoubtedly contained the double recessives. When a close examination of the individual lines in the Colsess crosses was made a variation was noticed in the number of xantha and white plants. Some lines were higher in white seedlings and others were

higher in xantha seedlings. When all lines are grouped a difference of 44 seedlings in favor of the xantha class out of a total of 10954 white and xantha seedlings was found. In the determination of the agreement between the calculated and observed ratios the double recessives have been considered as being included in the white class. It will be noted from table 13 that the fit of the observed ratio to the calculated 9:3:4 ratio is poor. $X^2 = 565.44$. Since the double recessive group cannot be separated from the white seedlings of the genetic constitution $a_c a_c X_c X_c$ and $a_c a_c X_c x_c$ it is not possible to use a formulae based on the double recessive group to determine the linkage value. Various crossover values were calculated and the different ratios compared with the observed. From the calculation it was found that the white class which contained the double recessive chlorophyll classes and simple recessive white group remained uniform for all degrees of linkage postulated. The X^2 method was used to determine the goodness of fit and good fits were obtained for crossover values between 12.5 and 4 percent.

TABLE 14

The observed and calculated ratio for various crossover percentages.

	CROSSOVER PER CENT	GREEN	XANTHA	ALBINO	X^2	P
Observed		11183	5499	5455		
Calculated	*50*	*12546*	*4182*	*5576*	*565.44*	*.0000*
"	*20*	*11375.0*	*5352.96*	*5576*	*9.8520*	*.0074*
"	*12.5*	*11239.12*	*5488.87*	*5576*	*2.9232*	*.2343*
"	*12*	*11232.33*	*5495.63*	*5576*	*2.8458*	*.2455*
"	*11.1*	*11220.92*	*5507.20*	*5576*	*2.7550*	*.2587*
"	*10*	*11207.76*	*5520.24*	*5576*	*2.7621*	*.2576*
"	*9.1*	*11198.08*	*5529.29*	*5576*	*2.8189*	*.2494*
"	*8.3*	*11190.66*	*5537.24*	*5576*	*2.8938*	*.2386*
"	*4.76*	*11164.65*	*5563.36*	*5576*	*3.3738*	*.1903*
"	*4.*	*11160.92*	*5567.08*	*5576*	*3.4602*	*.1827*

Further studies were made using the formulae presented by Collins (1) for use when there is difficulty in distinguishing any two classes. The formulae used were $p = \sqrt{\frac{AB - 2Ab}{AB + Ab}}$ and $p = \sqrt{\frac{4AB - 2n}{n}}$. When p is calculated from the ratio of AB to Ab[1] its value is 0.1053. When p is calculated from the ratio of AB to the total it equals 0.0745. When the above

[1] AB, Ab, aB, ab = the number of individuals in each of the four zygotic classes in a hybrid involving two character pairs Aa and Bb.

results are considered as crossover percentages values of 10.53 and 7.45 are obtained.

To further test the linkage relationship, the number of differently breeding F_3 lines which would be expected from the green plants of the F_2 families which segregated into green, xantha and white seedlings was calculated for independent inheritance and various degrees of linkage and compared with the observed ratio. The calculations were made from the following relationships based on Emerson's gametic ratio r:s

$$\begin{aligned} AA\ BB &= r^2 \\ AA\ Bb &= 2rs \\ Aa\ BB &= 2rs \\ Aa\ Bb &= 2s^2+2r^2 \end{aligned}$$

The calculated ratio of F_2 plants in each of the 4 genotypic groups AA BB, AA Bb, Aa BB, and Aa Bb on the basis of independent inheritance is 1:2:2:4; for 10 percent crossing over 1:18:18:164 and for 4 percent crossing over 1:48:48:1154. Considering each of the middle classes as one, the ratios become: 0.5:1:1:2; 0.055:1:1:9.1; 0.0:1:1:24.04. When we compare the above ratios with the observed breeding behavior of F_3 families all 47 of which segregated for green, xantha, and white, it appears that the crossover value is less than 4 percent. In order to determine the value more accurately, larger numbers are needed. The above method seems to offer possibilities for testing the crossover percentage for crosses in which the double recessive cannot be separated and where the recessive and double recessive plants are lost before maturity. It appears very probable, however, that the factor pair $X_c x_c$ for green vs. xantha seedlings in Colsess is closely linked to the factor pair $A_c a_c$ for green versus white seedlings in Colsess. The crossover percentage is low.

White seedlings in Trebi with xantha seedlings in Colsess

Crosses were made between Trebi I which carries the factor pair $A_t a_t$ and Colsess IV which carries a factor pair for xantha seedlings $X_c x_c$. In the F_1 generation 31 seedling plants were pure green from crosses where both parents were known to be heterozygous for their respective chlorophyll deficient seedling factors indicating that white seedlings in Trebi and xantha seedlings in Colsess IV are caused by the interaction of genetic factors which are not allelomorphic. The progeny of twenty-nine F_1 plants were studied. Seven segregated for green, xantha, and white seedlings; 9 segregated for green and xantha seedlings; 5 segregated for green and white seedlings and 8 bred true for pure green seedlings. The F_1 plants

were very small and only a few seeds were obtained from each. In order to study the segregations with a larger number of seedlings, the progeny from the green plants from lines which segregated in F_2 for three types of seedlings were grown in F_3. The breeding behavior of the progeny of 32 F_2 plants is given in table 15.

TABLE 15

F_3 breeding behavior of the progeny of the green plants from the F_2 families segregating in a 9:3:4 ratio.

13 Lines segregating in a 9:3:4 ratio

OBSERVED RATIO			*Calculated Ratio*			
Green	Xantha	White	*Green*	*Xantha*	*White*	X^2
2872	848	1065	*2691.6*	*897.2*	*1196.2*	*29.18*

7 Lines segregating in a 3:1 ratio of green to xantha

OBSERVED RATIO		*Calculated Ratio*		DEV. IN NUMBERS	$\frac{D}{PE}$
Green	Xantha	*Green*	*Xantha*		
1730	560	*1717.5*	*572.5*	12.5	0.89

6 Lines segregating in a 3:1 ratio of green to white

OBSERVED RATIO		*Calculated Ratio*		DEV. IN NUMBERS	$\frac{D}{PE}$
Green	White	*Green*	*White*		
1910	631	*1905.75*	*635.25*	4.25	0.29

6 lines produced only pure green seedlings

Any linkage, if present, would be studied in the 13 lines segregating for green, xantha, and white seedlings. If linkage were present it would be of the repulsion phase as in the previous cross. With linkage in the repulsion phase, the number of individuals in the middle or phenotypic classes Ab and aB would be high and the extreme classes or AB and ab would be low. In this cross the AB class is represented by the green seedlings and the Ab class by the xantha seedlings. The aB and ab classes or white seedlings are grouped and cannot be differentiated. Therefore with linkage in the repulsion phase the xantha seedlings should be higher than for independent inheritance and the green seedlings lower than calculated for independent inheritance. As this is not the case, however, as shown by the results given in table 15 it may safely be concluded that the

factor pairs $A_t a_t$ and $X_c x_c$ are inherited independently. The agreement with a 9:3:4 ratio calculated for independent inheritance is not good. The number of chlorophyll deficient seedlings of both types are lower than the calculated. A certain amount of elimination of the seedlings which are recessive for chlorophyll deficiencies might explain this condition.

Further tests were made with the lines segregating in F_2 for green versus xantha and those segregating for green versus white seedlings. Progeny from heterozygous F_2 plants were grown in the F_3 and seedling counts made. The data are given in table 16.

TABLE 16

Summary of segregation for xantha and white seedlings in lines heterozygous for a single factor pair.

SEGREGATING FOR	OBSERVED		Calculated		DEVIATION IN NUMBERS	D / PE
	Green	Chlorophyll defective	*Green*	*Chlorophyll defective*		
$C_c x_c$	1353	366	*1289.25*	*429.75*	63.75	5.28
$A_t a_t$	3126	973	*3074.25*	*1024.75*	51.75	2.77

The number of xantha seedlings is lower than the calculated with a value of D/PE of 5.28.

INTERRELATIONS OF CHARACTER PAIRS FOR OTHER BOTANICAL CHARACTER DIFFERENCES

Black versus white glume color (Bb) and hoods versus awns (Kk)

Studies were made in crosses between Colsess×*H. distichon nigrinudum*, Colsess×Minnesota 90–5, and Colsess×Minnesota 90–8. The segregations obtained in the F_2 generation are given in table 17.

TABLE 17

Segregation in F_2 for black versus white glumes (Bb) and hoods versus awns (Kk) in crosses between Colsess ×H. distichon nigrinudum, Colsess×Minnesota 90–5 and Colsess×Minnesota 90–8.

CROSS		*BK*	*Bk*	*bK*	*bk*	X^2	*P*
Colsess×*H. dis. nig.*	obs.	2611	920	860	332	7.45	.0602
	cal.	*2657*	*886*	*886*	*295*		
Colsess×Minn. 90–5	obs.	603	182	194	61	1.67	.6479
	cal.	*585*	*195*	*195*	*65*		
Colsess×Minn. 90–8	obs.	166	58	63	25	2.41	.4983
	cal.	*175.5*	*58.5*	*58.5*	*19.5*		

In the cross between Colsess×*H. distichon nigrinudum* the number of awned plants in both the black and white glumed classes is higher than calculated on a 3:1 basis which explains the X^2 value of 7.45 with a P value of .0602. The chances that a deviation as high or higher may occur due to random sampling are 602 in 10000 trials or about 1 in 17. The other two crosses fit the expected ratio for independent inheritance very well. It can safely be concluded from the above data that black versus white glume color is caused by a factor pair inherited independently of the factor pair for hoods *versus* awns.

Black versus white glume color (Bb) and long versus short haired rachillas (Ss)

The results of three crosses, Colsess×Minnesota 90–5, Colsess×Minnesota 90–8, and Trebi×Minnesota 90–8 are given in table 18.

TABLE 18

Segregation in F_2 for black versus white glumes (Bb) and long versus short haired rachillas (Ss).

CROSS		*BS*	*Bs*	*bS*	*bs*	X^2	*P*
Colsess×Minn. 90–5	obs.	592	193	201	54	2.14	.5471
	cal.	*585*	*195*	*195*	*65*		
Colsess×Minn. 90-8	obs.	164	60	74	14	6.45	.0937
	cal.	*175.5*	*58.5*	*58.5*	*19.5*		
Trebi×Minn. 90–5	obs.	520	154	147	48	4.88	.1826
	cal.	*484.3*	*161.4*	*161.4*	*53.8*		

From the above data it is concluded that the factor pair for black and white glume is inherited independently of the factor pair which differentiates long *versus* short haired rachillas.

Black versus white glume color (Bb) and covered versus naked seed (Nn)

The factor pairs concerned were *Bb* and *Nn*. The results are given in table 19.

TABLE 19

Segregation in F_2 for black versus white glumes (Bb) and covered versus naked seed (Nn) in a cross between Colsess and H. distichon nigrinudum.

	BN	*Bn*	*bN*	*bn*	X^2	*P*
Observed	2679	852	914	279	3.26	.3578
Calculated	*2657*	*886*	*886*	*295*		

These data indicate that the factor pair for black *versus* white glume

color is inherited independently of the factor pair for covered *versus* naked seed.

Black versus white glume color (Bb) and non-six-rowed versus six-rowed (Vv)

Studies were made of the factor pairs *Bb* and *Vv* in a cross between Colsess and *H. distichon nigrinudum*. The F_2 plants were classified as six-rowed and non-six-rowed. Table 20 presents the data obtained in F_2.

TABLE 20

Segregation in F_2 for non-six-rowed versus six-rowed (Vv) and black versus white glume color (Bb).

	BV	*bV*	*Bv*	*bv*	X^2	*P*
Observed	304	108	88	37		
Calculated	*302*	*100.7*	*100.7*	*33.6*	*2.48*	*.4856*

BV = Black glume non-six-rowed
bV = White glume non-six-rowed
Bv = Black glume six-rowed
bv = White glume six-rowed

From the above data it may be concluded that the factor pair *Bb* for black *versus* white glume is inherited independently of the factor pair *Vv* for non-six-rowed *versus* six-rowed.

Hoods versus awns (Kk) and long versus short haired rachilla (Ss)

Studies were made in crosses between Colsess×*H. deficiens nudideficiens*, Colsess×Minnesota 90–5, and Colsess×Minnesota 90–8. Table 21 gives

TABLE 21

Segregation in F_2 for hoods versus awns and long versus short haired rachilla.

CROSS		*KS*	*Ks*	*kS*	*ks*	X^2	*P*
Colsess×*H. def. nud.*	obs.	1983	667	753	234	9.87	.0198
	cal.	*2046*	*682*	*682*	*227*		
Colsess×Minn. 90–5	obs.	609	188	184	59	2.40	.4820
	cal.	*585*	*195*	*195*	*65*		
Colsess×Minn. 90–8	obs.	175	54	63	20	0.71	High
	cal.	*175.5*	*58.5*	*58.5*	*19.5*		

the observed and calculated ratios in the F_2 generation for hoods *versus* awns and long *versus* short haired rachilla. In the cross between Colsess and *H. deficiens nudideficiens* there is a wide deviation between the mono-

hybrid observed and calculated ratio for hoods *versus* awns. The awned group is higher than calculated and X^2 for a 9:3:3:1 ratio is 9.87. For this cross a calculated crossover value of 48.88 percent was obtained by Yule's coefficient of association Q (1924). A crossover value as high as this cannot be distinguished from independent inheritance with any degree of certainty in an F_2 generation. The value for P for the two other crosses indicates independent inheritance of the factor pairs for hoods *versus* awns and long *versus* short haired rachilla.

Hoods versus awns (Kk) and covered versus naked seed (Nn)

In crosses between Colsess×*H. distichon nigrinudum* and Colsess×*H. deficiens nudideficiens* a study was made of the interrelations of the factor pairs for hoods *versus* awns, Kk, and covered versus naked seed, *Nn*. Table 22 presents the F_2 data.

TABLE 22

Segregation in F_2 for hoods versus awns and covered versus naked seeds.

cross		*KN*	*Kn*	*kN*	*kn*	X^2	*P*
Colsess×*H. dis. nig.*	obs.	2635	836	958	294	8.88	.0313
	cal.	*2657*	*886*	*886*	*295*		
Colsess×*H. def. nud.*	obs.	1969	681	737	250	9.60	.0229
	cal.	*2056*	*682*	*682*	*227*		

In the above two crosses the awned plants are higher than calculated on a 3:1 basis which causes the dihybrid segregation to deviate widely from a calculated 9:3:3:1 ratio. The crossover value as obtained by the use of Q is 49.35 percent for the first cross and 49.20 percent for the second cross. The above crossover values cannot be considered as indications of linkage. However X^2 for the goodness of fit to a calculated 9:3:3:1 is 8.88 for the first cross and 9.50 for the second. P is .0313 and .0240 respectively. It can be concluded that the factor pair which causes the production of hoods versus awns is inherited independently of the factor pair which differentiates covered versus naked seeds.

Hoods versus awns (Kk) and non-six-rowed versus six-rowed (Vv)

In a cross between Colsess and *H. distichon nigrinudum* the interrelations were studied of the factor pairs, *Kk* for hoods *versus* awns and *Vv* non-six-rowed *versus* six-rowed. The F_2 plants were classified as six-rowed or non-six-rowed. In table 21 the different F_2 phenotypes are

represented by letters as follows: *KV* is hooded and non-six-rowed; *kV* is awned and non-six-rowed; *Kv* is hooded and six-rowed, and *kv* is

TABLE 23

Segregation in F_2 for hoods versus awns (Kk) and six rowed versus non-six-rowed (Vv).

	KV	*kV*	*Kv*	*kv*	X^2	*P*
Observed	320	92	97	28	2.89	.4115
Calculated	*302*	*100.7*	*100.7*	*33.6*		

awned and six-rowed. The above data indicate that the factor pair for hoods *versus* awns is inherited independently of the factor pair for six-rowed *versus* non-six-rowed.

Long versus short haired rachilla (Ss) and covered versus naked seed (Nn)

Studies were made of the interrelations of the factor pairs *Ss* and *Nn* in a cross between Colsess and *H. deficiens nudideficiens*. The F_2 data are presented in table 24.

TABLE 24

Segregation in F_2 for long versus short haired rachilla and covered versus naked seed.

	SN	*Sn*	*sN*	*sn*	X^2	*P*
Observed	2061	675	645	256	5.79	.1242
Calculated	*2046*	*682*	*682*	*227*		

There is some deviation from the calculated 9:3:3:1 ratio. The genotypes of the parents of this cross were *NNss* and *nnSS*; therefore if linkage was present the *SN* and *sn* classes should have a similar number than is expected with independent inheritance which is not the case. The calculated value of *p* by Yule's Coefficient of Association was .4730. On these bases the conclusion may be made that the factor pair *Ss* for long and short haired rachilla and the factor pair *Nn* for covered and naked seed are inherited independently.

Covered versus naked seed (Nn) and non-six-rowed versus six-rowed (Vv)

The factor pairs *Nn* and *Vv* were studied in a cross between Colsess and *H. deficiens nudideficiens*. Table 25 presents the F_2 data for covered *versus* naked seed and non-six-rowed *versus* six-rowed.

TABLE 25

Segregation in F_2 for covered versus naked seed and non-six-rowed versus six-rowed.

	VN	*Vn*	*vN*	*vn*	X^2	*P*
Observed	315	97	94	31	1.35	.7212
Calculated	*302*	*100.7*	*100.7*	*33.6*		

The data in table 25 indicate that the factor pair *Nn* is inherited independently of the factor pair *Vv*.

INTERRELATIONS OF CHLOROPHYLL DEFICIENCIES WITH OTHER BOTANICAL CHARACTERS

Green versus white seedlings (A_1a_1) and black versus white glume color (Bb)

In a cross between Trebi and Minnesota 90–5 the factor pairs studied were in the coupling phase. The Trebi parent was white glumed and carried the factor for white seedlings. The Minnesota 90–5 parent was black glumed and was homozygous for green seedlings. The F_2 segregation for green *versus* white seedlings and black *versus* white glumes is given in table 26.

TABEL 26

Segregation in F_2 for green versus white seedlings and black versus white glume color.

	A_1B	A_1B	a_1B—a_1b	X^2	*P*
Observed	447	68	157	40.01	very low
Calculated (9:3:4)	*378*	*126*	*168*		

The white seedling plants died and only the green plants survived to maturity. It will be observed that the white glumed class has fewer plants than are expected with independent inheritance and the fit to a calculated 9:3:4 ratio is very poor. Two of the classes were eliminated by the death of the white seedlings. To calculate the linkage value: Collins' formula $p=\sqrt{\frac{AB--2Ab}{AB+Ab}}$ in which the crossover value is calculated from two classes was used. The crossover percentage obtained by this method was 22.29 percent. The observed data are compared with the theoretical on the basis of a crossover value of 22.29 percent (see table 27).

TABLE 27

Comparison of observed and calculated on the basis of a C. O. value of 22.29 per cent for the characters green versus white seedlings (A_ta_t) black versus white glumes in a cross between Trebi and Minnesota 90–5.

	A_tB	A_tb	a_tB——a_tb	CALCULATED CROSSOVER PERCENT	X^2	P
Observed	447	68	157	22.29	1.00	.6065
Calculated	*436*	*68*	*168*			

The agreement between observed and calculated was good and apparently the factor pair A_ta_t and Bb are linked.

Green versus white seedlings (A_ca_c) and black versus white glume color (Bb)

Crosses between Colsess×*H. distichon nigrinudum*, Colsess×Minnesota 90–5, and Colsess×Minnesota 90–8 were studied. Table 28 gives the observed and calculated ratio for the factor pairs concerned.

TABLE 28

Segregation in F_2 of green versus whit seedlings A_ca_c and black versus white glume color.

CROSS		A_cB	A_cb	a_cB——a_cb	X^2	P
Colsess×*H. dis. nig.*	obs.	1953	690	812	5.88	.0537
	cal.	*1943*	*648*	*864*		
Colsess×Minn. 90–5	obs.	1283	408	518	3.53	.1766
	cal.	*1243*	*414*	*552*		
Colsess×Minn. 90–8	obs.	434	178	181	7.60	.0231
	cal.	*446*	*149*	*198*		

In this case as in other crosses the number of white seedlings is lower than calculated on a 3:1 basis. As no striking deviation is found in the other two classes it is evident that the factor pair for black *versus* white glume color is inherited independent of the A_ca_c factor pair for chlorophyl development. It will be remembered that the factor pair in Colsess for green *versus* white seedlings, A_ca_c, was independent of that in Trebi, A_ta_t, and that A_ta_t from Trebi was linked with a factor pair (Bb) for black *versus* white glumes.

Green versus white seedlings (A_ca_c) and hoods versus awns (Kk)

Studies were made in crosses between Colsess×*H. distichon nigrinudum*, Colsess×Minnesota 90–5 and Colsess×Minnesota 90–8. The cross was

made in the repulsion phase. If linkage is present the a_cK and A_ck classes would be larger than for independent inheritance. The plants having the recessive condition a_ca_c would die in the seedling stage. With simple Mendelian independent inheritance and an absence of linkage a 9:3:4 ratio would be expected. Table 29 presents the expected and calculated ratios in F_2.

TABLE 29

Segregation in F_2 for the characters green versus white seedlings and hoods versus awns.

		A_cK	A_ck	a_cK—a_ck	X^2	P
Colsess×*H. dis. nig.*	obs.	1939	704	812	7.98	.0185
	cal.	*1943*	*648*	*864*		
Colsess×*H. def. nud.*	obs.	1053	397	479	4.43	.1135
	cal.	*1085*	*362*	*482*		
Colsess×Minnesota 90–5	obs.	1269	422	518	2.84	.2463
	cal.	*1243*	*414*	*552*		
Colsess×Minnesota 90–8	obs.	435	175	181	6.43	.0405
	cal.	*446*	*149*	*198*		

The above data gives a fair fit to the calculated ratio 9:3:4. There is a slightly higher number of individuals in the green awned class and a slightly lower number in the green hooded class than is expected with independent inheritance. This slight deviation is persistent in all four crosses and suggests a linkage with a high percentage of crossing over.

TABLE 30

F_2 segregations for green versus white seedlings and hoods versus awns and calculated linkage values for the different crosses.

CROSS	A_cK	A_ck	a_cK—a_ck	*Calculated Crossover Percent*
Colsess×*H. dis. nig.*	1939	704	812	44.82
Colsess×*H. def. nud.*	1053	397	479	42.26
Colsess×Minnesota 90–5	1269	422	518	50.13
Colsess×Minnesota 90–8	435	175	181	37.32
Total	4696	1698	1990	45.09

The calculated linkage value was obtained from Collins' formulae $p=\sqrt{\frac{AB-2Ab}{AB+Ab}}$ for use when there is difficulty in distinguishing any two

classes. Considerable variation was found in the calculated percentage of crossing over in the different crosses. The white seedling class is lower than calculated on a 3:1 basis and there is evidently some gametic elimination of the white seedling classes. The calculated results for independent inheritance was obtained from a gametic ratio for the factors for green and white seedlings of 1.0525 to 1. This ratio was calculated from the phenotypic ratio of green to white seedlings of 3.213:1 which was obtained from these crosses. In comparing observed with theoretical on the basis of 45.09 crossing over it was considered also that the functional gametes bearing the factors for green and white seedlings were in the ratio of 1.0525 to 1. The X^2 value for the linkage with 45.09 percent crossing over is .01 and P is very large while the X^2 value for independent inheritance is 8.17 with a P value of .0042 (see table 31).

TABLE 31

Observed and calculated ratios for independent inheritance and 45.09 percent crossing over.

RATIO	A_cK	A_ck	a_cK——a_ck	X^2	P^*
Observed	4696	1698	1990		
Independent inheritance	*4795*	*1599*	*1990*	8.17	.0042
45.09 percent crossing over	*4692*	*1702*	*1990*	.01	very large

* P was obtained from table of X^2 where n = 2.

Green versus white seedlings (A_ca_c) and long versus short haired rachilla (Ss)

Studies were made in crosses between Colsess × *H. deficiens nudideficiens*, Colsess × Minnesota 90–5 and Colsess × Minnesota 90–8. Table 32 presents the F_2 segregation for green *versus* white seedlings and long *versus* short haired rachilla.

While some slight deviations from the calculated 9:3:4 ratio were found, no consistent variation was observed except for the classes of white seedlings which were lower than calculated in each case. The X^2 for the crosses between Colsess and the Minnesota selections was 4.02 and 5.61 with values of .1353 and .0624 respectively. In the cross between Colsess and *H. deficiens nudideficiens* the value of X^2 is 0.80, which gives a value of P which is very large. These data indicate that the factor pair A_ca_c for green and white seedlings is inherited independently of the factor pair for long and short haired rachilla.

TABLE 32

Segregation in F_2 for green versus white seedlings and long versus short haired rachillas in crosses between Colsess×H. deficiens nudideficiens, Colsess×Minnesota 90–5 and Colsess× Minnesota 90–8.

CROSS		A_cS	A_cs	a_cS—a_cs	X^2	P
Colsess×*H. def. nud.*	obs.	1073	377	479	0.80	large
	cal.	*1085*	*362*	*482*		
Colsess×Minn. 90–5	obs.	249	442	518	4.02	.1353
	cal.	*1243*	*414*	*552*		
Colsess×Minn. 90–8	obs.	479	133	181	5.61	.0624
	cal.	*446*	*149*	*193*		

Green versus white seedlings (A_ta_t) and long versus short haired rachilla (Ss)

Studies were made in a cross between Trebi and Minnesota 90–5. The results are given in table 33.

TABLE 33

Segregation in F_2 for green versus white seedlings (A_ta_t) and long vs. short haired rachilla (Ss).

	A_tS	A_ts	a_tS—a_ts	X^2	P
Observed	388	127	157	0.98	large
Calculated	*378*	*126*	*168*		

The observed ratio fits the calculated 9:3:4 ratio very well, X^2 being 0.98. This indicates clearly that the factor pair A_ta_t is inherited independently of the factor pair *Ss*.

Green versus white seedlings (A_ca_c) and covered versus naked seed (Nn)

The inheritance of the interrelations of green *versus* white seedlings and covered *versus* naked seeds were studied in crosses between Colsess ×*H. distichon nigrinudum* and Colsess×*H. deficiens nudideficiens*. In the above crosses one parent was homozygous for the green seedling and naked seed factors and the other heterozygous for the factor pair for green *versus* white seedlings and homozygous for the covered seed factor. The F_2 segregation agrees well with the calculated ratio of 9:3:4, X^2 being 4.23 for the cross Colsess×*H. distichon nigrinudum* and 0.51 for the Colsess ×*H. deficiens nudideficiens* cross. The calculated value of P was .1231 for the former and very large for the latter cross. (See table 34.)

TABLE 34

Segregation of F_2 for green versus white seedlings and cover versus naked seed.

CROSS		A_cN	A_cn	a_cN—a_cn	X^2	P
Colsess×*H. dis. nig.*	obs.	1975	668	812	4.23	.1231
	cal.	*1943*	*648*	*864*		
Colsess×*H. def. nud.*	obs.	1076	374	479	0.51	large
	cal.	*1085*	*362*	*482*		

It is evident from the above data that the factor pair A_ca_c is inherited independently of the factor pair *Nn*.

Green versus white seedling (A_ca_c) and non-six-rowed versus six-rowed (Vv)

This study of interrelations was made in a cross between Colsess×*H. distichon nigrinudum*. Table 35 gives the F_2 segregation for green *versus* white seedlings and non-six-rowed *versus* six-rowed.

TABLE 35

Segregation of F_2 for green versus white seedlings and non-six-rowed versus six rowed.

	A_cV	A_cv	a_cV—a_cV	X^2	P
Observed	483	141	195	2.58	.2839
Calculated (9:3:4)	*460.7*	*153.6*	*204.8*		

The data presented in table 35 clearly indicate that the factor pair A_ca_c is inherited independently of the factor pair *Vv*.

SUMMARY

1. Simple Mendelian inheritance was found for the following factor pairs: black *versus* white glume color (*Bb*), hoods *versus* awns (*Kk*), covered *versus* naked seed (*Nn*), non-six-rowed *versus* six-rowed (*Vv*), long *versus* short-haired rachilla (*Ss*), green *versus* white seedlings (A_ca_c), green *versus* white seedlings (A_ta_t) and green *versus* xantha seedlings (X_cx_c).

2. A two-factor difference was found for fertility of the lateral florets in crosses involving Colsess, a six-rowed barley, and *H. deficiens nudideficiens*, Colsess × Minnesota 90–5. Colsess evidently has the genetic constitution *vv* II while *H. deficiens nudideficiens* and Minnesota 90–5 the genetic constitution *VV* ii.

3. Three genetically different seedling chlorophyll deficiencies are inherited as simple Mendelian recessives. These chlorophyll deficiencies are designated as follows: white a_ta_t, white a_ca_c, and xantha x_cx_c.

4. Two complementary factors for chlorophyll production were found in a strain of Colsess. One factor pair was found to be $A_c a_c$; further studies will be made with the other pair.

5. Independent Mendelian inheritance was found between the following character pairs: black versus white glume color, hoods versus awns, covered versus naked seed, non six-rowed versus six-rowed, and long versus short-haired rachilla.

6. Studies of the interrelation of chlorophyll deficiencies and other characters indicate two probable linkage groups:

(a) Green *versus* white seedlings $A_c a_c$, green *versus* xantha seedlings $X_c x_c$, and possibly hoods *versus* awns Kk.

(b) Black *versus* white glume color Bb and green *versus* white seedlings $A_t a_t$.

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ACQUIRED IMMUNITY IN PLANTS

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INTRODUCTION

Certain phenomena observed in the development of the embryos of various Nicotiana species crosses led to the present investigation. The embryos produced in the crosses *Nicotiana rustica*×*Nicotiana Rusbyi*, *Nicotiana rustica*×*Nicotiana glauca*, *Nicotiana rustica*×*Nicotiana alata* *Nicotiana rustica*×*Nicotiana Langsdorffii*, *Nicotiana rustica*×*Nicotiana Tabacum*, etc., do not reach the normal size produced by selfing or by mating two plants belonging to the same species. In the first two combinations, the embryos grow from 6 to 10 days, depending upon the environmental conditions; the development then stops and the embryos die within a short time. In the other combinations mentioned the embryos reach a higher stage of development, though they are always behind the normal stage. Development in the majority of these hybrids stops within 15 to 20 days, an exceptional few producing viable seeds. A conspicuous feature in such cases is that a great amount of starch accumulates in the integumental tissue outside of the nucellus which envelops the hybrid embryo and endosperm.

These and some other phenomena, which will be mentioned later, give rise to the question: What is the inhibitor of embryo development?

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Are antibodies produced after interaction and reaction between the embryo and the endosperm (of hybrid nature) on the one hand, and the neighboring tissues of the mother plant on the other hand? In other words, are these reactions similar to the reactions produced by the so-called "*Abwehrfermente*" acting between the mother and the placenta, studied by ABDERHALDEN (1912a, b, c, 1913, etc.) in mammalia and determined by dialysis, ninhydrin reaction and polarization? Or, speaking more generally, is there a lytic reaction, preceded probably by a precipitation *in vivo*? No satisfactory answer can be given to this question until another question is solved, namely: Is the plant organism as well as the animal organism able to produce agents (Abwehrfermente, antibodies) which can attack foreign substances? This is the question we hope to answer here.

A brief historical review of the immunological literature treating questions connected with the present work will be given before the experimental work is described.

Historical

When certain foreign substances are parenterally introduced into the animal body, the physiological balance is disturbed. The organism reacts against the invader by the production of agents usually called "antibodies."

The first experimental data demonstrating the production of antibodies in the animal organism appeared near the end of the last century. The earliest observation in what one may call modern immunology, showed that the blood has the power to destroy bacteria. NUTTAL (1888) found that normal defibrinated blood when incubated with tissue containing anthrax bacilli brought about a reduction in the number of bacilli. BUCHNER (1889) pointed out the resemblance of the behavior of the active substance in such reactions to the behavior of enzymes, and called it alexin (protective substance). At about the same time the agglutination phenomenon was discovered by CHARRIN and ROGER (1889) by experimenting with the serum of rabbits rendered immune to *Bacillus pyocyaneus*. These observations were later confirmed by METCHNIKOFF (1891), KRUSE and PANSINI (1892), ISAEFF (1893), ISAEFF and IWANOFF (1894), and many others with different bacteria; but the principal significance of agglutination as a specific immunity phenomenon was studied by GRUBER and DURHAM (1896). At about the same time, agglutinations were found to be characteristic not only of many bacteria but also of red blood corpuscles (BORDET 1896, LANDSTEINER 1899, von DUNGERN

1899), leucocytes (LESCHKE 1913, REESER 1913), spermatozoa (LANDSTEINER 1908), algae (ROSENBLAT-LIECHTENSTEIN 1912, 1913) etc.

The discovery by RUDOLPH KRAUS (1897) that bacterial filtrates produce *precipitates* when mixed with the specific antiserum was a logical outcome of the discovery of agglutinins, and was followed two years later by the observation that this is a general reaction for other than bacterial proteins. TCHISTOWITCH (1889) secured precipitins for eel serum, BORDET (1899) for milk and chicken blood, and later KOWARSKY (1901), JACOBY (1901), MAGNUS and FRIEDENTHAL (1906), MAGNUS (1908), GASIS (1908), and others for plant proteins, while KARASAWA (1910) and GALLI-VALERIO et BORNAND (1912) caused anaphylactic shock with plant antigens.

While immunological investigations have been chiefly directed toward the perfection of methods for application to human therapy and to legal medicine, nevertheless various investigators, including UHLENHUTH (1900, 1901), WASSERMANN (1903), NUTTAL (1902), used the immunological reaction for differentiation of various species of animals. Moreover, the MEZ school, by methodical work for 18 years, has recently drawn a phylogenetical tree of the plants serologically (ZIEGENSPECK 1925a, 1925b, MEZ and ZIEGENSPECK 1926) from about 400 reaction centers. ZADE (1914), also working serologically with certain Leguminosae and Graminae, has even differentiated between varieties.

The work of the MEZ school, often called the "Königsberg school," has recently been reëxamined and questioned at Berlin in the GILG-SCHÜRHOFF laboratory. From the papers appearing thus far, it is obvious that the Berlin serologists do not doubt the specificity of the plant antigens ("Das von mir gewonene Serum war in allen Fällen spezifisch, d.h. es reagierte stets mit dem zum Impfen verwandten Samenauszuge am weitesten"—W. NAY 1927, p. 151). They attack the MEZ method, however, and emphasize the errors which may occur when working with heterogenetic serum which is obtained very frequently.

Both the Berlin and the Königsberg schools used plants for obtaining antigens, while for antibody production the rabbit's body was usually utilized.

It has long been known that many of the warm blooded animals, and even some mollusks, are able to produce antibodies. The question of the production of antibodies in plants, however, has been neglected. The meagre literature treating of this question usually confines discussion to activity of normal precipitins and agglutinins of plant origin on the blood sera and

red blood corpuscles. Thus LANDSTEINER and RAUBITSCHEK (1908) precipitated horse and fowl proteins by extracts of Phaseolus, Pisum, Vicia, and Lens. The same extracts agglutinated the red blood corpuscles of horse and fowl. von Eisler and von Portheim (1909) found in Datura agglutinins against horse and rabbit red corpuscles and precipitins against horse and rabbit sera. They have shown by these experiments the coëxistence of normal agglutinins and precipitins. Precipitin and agglutinin reactions on sera and red blood corpuscles were also produced by ricin, abrin and crotin (WILENKO 1910), by extracts of rice (KANAHARA 1912), of *Zea mays* (LUI and BACELLI 1913), by phaseolin, phaselin, and bean albumin (SCHNEIDER 1912), by extracts of *Cotyledon Scheideckeri* (KRITCHEWSKY 1914) and by extracts of many fruits, roots and leaves (CARBONE ed VIGLIANO 1922). Agglutination of bacteria was observed by WAGNER (1915) in the juice of *Solanum tuberosum* and by KOŘINEK (1924) in the juice of *Beta vulgaris*.

Recently J. RODRIGUEZ SARDIÑA (1926, p. 303), investigating the antibody production in plants by injection of bacteria, came to the following negative conclusion.

"Es kann also auf Grund der bis jetzt durchgeführten Untersuchungen gesagt werden, dass sich artspezifische Agglutinine und Precipitine . . . . bei Opuntia, Kartoffeln, Tomaten, Kurbis und Vicia Faba nicht bilden und dass sich dennach die Pflanzen in dieser Hinsicht anders verhalten als Tiere und Menschen."

The negative results obtained by SARDIÑA probably are due to the small quantity of the injected antigen. The mechanical constitution of the plant organism differs so materially from that of the animal that it is usually impossible to inject a large quantity of antigen, and even difficult to inject the amount necessary for the induction of antibodies in sufficient quantity for their effects to be observed *in vitro*. When one considers the ratio of the proteins, carbohydrates, water, etc., and the reaction ability of the cells in plants and in animals (MÜLLER 1926), it is obvious that for acquiring immunity in plants one ought to deal in larger amounts of antigen. Since the introduction of sufficient amount of antigen in plants by injection is impossible, one has to apply some other method. We hope we have succeeded by intergrafting certain solanaceous plants. In a graft union the constituents of sap exchanged mutually between scion and stock act as do injected antigens in rabbits.

The technical terminology to be used in the present paper is given in the following paragraph.

Terminology

The word "immunity" includes three different phenomena. A parasite may not attack certain organisms because they may have a covering layer or layers of tissue impenetrable for the parasite in question. In this case we speak of "mechanical immunity." There are, for example, certain citrus-species (STRAPP 1927) which have mechanical immunity against *Pseudomonas citri*. The resistance of an organism against certain parasites may be due to the particular chemical substances which the organism contains, thus making it an unfavorable medium for the parasites. Such an immunity is usually called "physiological immunity." This may be "natural" or "normal" if the organism contains substances without any previous induction, as acid (Comes), alkaloids, etc., which prevent the thriving of the parasites, or "acquired" if the preventive substances are produced only after induction by agents of antigenic nature. An antigen is called any substance which is able to induce the production in an organism of reacting substances against the antigen itself. The preventive substances are called antibodies. In the present work we shall use the term "normal" or "antigenic" extract when an extract has been obtained from a plant in which no production of antibodies has been induced, and the term "immune" extract when the extract has been obtained from a plant in which production of antibodies was induced by grafting.

Acknowledgment

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MATERIAL

The following plants were used in the experiments:
Solanum tuberosum L., *Solanum Melongena* Mill., *Solanum Lycopersicum* L., *Solanum Dulcamara* L., *Solanum nigrum* L., *Physalis peruviana* L., *Salpiglossis sinuata* Rüiz et Pav., *Browalia speciosa* H., *Capsicum pyramidale* Mill., *Lycium Barbarum* L., *Datura ferox* Nees, *Datura Wrightii* Nort., *Petunia violacea* Lindl., and certain species of Nicotiana, namely: *Nicotiana Tabacum* L., *Nicotiana Rusbyi*, *Nicotiana rustica* L., *Nicotiana glauca* R. Gr., *Nicotiana Langsdorffi* Wm., *Nicotiana alata* Link and Otto,

Nicotiana Sanderae Hort., *Nicotiana longiflora* Cav., *Nicotiana suaveolens* Lehm., *Nicotiana quadrivalvis* Pursh, F_1 (*Nicotiana rustica* × *Nicotiana Tabacum*), F_1 (*Nicotiana rustica var. humilis* × *Nicotiana rustica var. brasila*), F_1 (*Nicotiana rustica* var. *humilis* × *Nicotiana rustica* var. *texana*) *and* F_1 (*Nicotiana Tabacum* × *Nicotiana Rusbyi.*)

All were grown in the greenhouse and in the garden of the BUSSEY INSTITUTION at HARVARD UNIVERSITY.

METHODS

Grafting

All of the methods of grafting described by WINKLER (1924) were tried; most frequently, however, simple whip grafting, tongue whip grafting, cleft grafting, saddle grafting, and budding were used. The best results were obtained by simple whip grafting (figure 1). The scion and the stock were bound together by common string 0.5–1.5 mm thick.

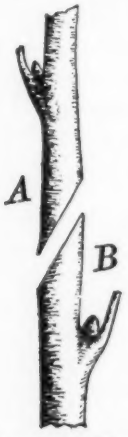

FIGURE 1.—Scion (A) and stock (B) prepared for grafting.

Better and faster union between scion and stock was obtained when the components were moistened by 1 percent water solution of $MgSO_4$. This increased the percentage of successful unions.

Absorption

In addition to the grafting, we have tried to induce the production of antibodies by the absorption method used by SHEWIRJEFF (1903),

MOKRZECKI (1903) and RUMBOLD (1915), and modified for our purposes. By this method we did not succeed in introducing a great amount of antigenic extract, and we did not obtain any positive results.

Testing the precipitin reaction

Uhlenhuth's ring method (1909) and MEZ's prescription (1924) were applied for testing the precipitin reaction. The plant tissues (leaves and stems) were crushed in a mortar and extracted by distilled water 2 hours. In applying Uhlenhuth's ring method the extracts were made in the ratio of 1 part of plant tissues to 2 parts of distilled water. This extract was used as "normal concentration" and was further diluted for testing the precipitation capacity.

Chloroform was used successfully for the preservation of the extracts. Toluol at the top and chloroform at the bottom were used for keeping extracts a longer time. The test tubes which were used for testing the precipitation capacity of extracts after Uhlenhuth's method were 3–4 mm in diameter and 30–35 mm long. For successful layering of both component extracts, pipettes of about 0.5 mm diameter were prepared. The tubes were boiled 2 hours in 0.5 percent solution of Na_2CO_3 and 4 hours in distilled water changed four times before using. The formation of Uhlenhuth's ring was observed usually for 40 minutes; sometimes it persisted even longer. In applying MEZ's prescriptions the material for both extract components was treated by: (a) tartaric acid-alcohol-ether; (b) ether, (c) chloroform or (d) chloroform-alcohol before the extraction. The extracts were obtained by taking an equal quantity of treated tissues and distilled water. The development of the reactions was observed for 24 hours. The precipitation capacity following the Uhlenhuth's ring method was tested after the treatment of the materials by the above mentioned chemical agents also.

A description of the experiments and the results obtained between the normal extracts precedes the description of the acquired precipitins.

NORMAL PRECIPITINS

It has been known for a long time that the serum of normal unimmunized animals often has the capacity to produce agglutinin and precipitin reactions when not too greatly diluted. Thus ASCOLI (1903) found that ox-serum contains precipitins for the sera of man, dog, pig, goat, rabbit, guinea pig, and fowl; dog-serum for the sera of fowl and guinea pig. GAETHGENS (1910) pointed out that the presence of the normal precip-

itins depends to a certain extent upon the age of the rabbit. The sera of 5-weeks rabbits usually do not contain normal precipitins, while the sera of many adult and old rabbits contain them. NOGUCHI (1903), LOEB (1921), CHARKOVITCH (1921) and others found normal precipitins in the sera of cold-blooded animals. CANTACUZÈNE (1912) succeeded in precipitating the normal sera of horse and rabbit by serum of *Eupagurus prideauxii*. He stated that the normal precipitins of *Eupagurus prideauxii* are very labile and that a temperature of 55°C destroyed them. LANDSTEINER and REICH (1907), HEUER (1922) and others believe that the normal precipitins are not specific and, contrary to CANTACUZÈNE, that they are more resistant than the antibodies that result from immunization. Concerning the rôle which the normal agglutinins play during the acquiring of immunity, BOISSEVAIN (1922) offered the hypothesis that specific immune agglutinins are merely nuclei of antigens charged with normal agglutinins.

It has already been mentioned in the introductory notes that the normal extracts of plant organs contain agents which precipitate the normal sera of many animals and agglutinate their red blood corpuscles. The data of mutual precipitations of the normal extracts of certain solanaceous plants investigated by the author are shown in the following tables: 1, 2, 3, 4, 5, 6, 7, 8, and 9.

The capacity of precipitin reactions was determined by the size and density of the precipitation ring (Uhlenhuth's ring) and recorded as: − (none), ± (trace), +, ++, +++, ++++, the last representing the greatest amount found. The most striking precipitin reactions are given in the tables mentioned.

TABLE 1

Normal precipitin reaction of Solanum nigrum extract.

AGAINST NORMAL EXTRACTS OF	THE POTENCY OF THE PRECIPITIN REACTION AFTER					
	1 min.	5 min.	10 min.	20 min.	30 min.	40 min.
Capsicum pyramidale	+	+++	++++	++++	+++	+++
Nicotiana glauca	−	+	+	−	−	−
Nicotiana rustica	±	++	++	+	−	−
Physalis peruviana[1]	−	+	++	++	+	±
Datura Wrightii	++++	++++	+++	+++	+++	++
Solanum Dulcamara	−	−	−	−	−	−
Salpiglossis sinuata	±	++	+++	++	++	+
Lycium Barbarum[2]	−	−	−	−	−	−

[1] After 30 minutes two lytic rings appeared at both sides of the precipitin ring.

[2] Simple lytic ring.

TABLE 2

Normal precipitin reaction of Physalis peruviana extract.

AGAINST NORMAL EXTRACTS OF	THE POTENCY OF THE PRECIPITIN REACTION AFTER					
	1 min	5 min.	10 min.	20 min.	30 min.	40 min.
Capsicum pyramidale[1]	−	−	−	−	−	−
Lycium Barbarum	−	−	−	−	−	−
Nicotiana rustica	±	+++	+++	++	++	+
Nicotiana glauca	−	++	++	++	+	+
Solanum Dulcamara	+++	++++	++++	++++	+++	+++
Solanum Melongena	−	−	−	−	−	−
Solanum nigrum[2]	−	+	++	++	+	±
Solanum tuberosum	−	−	−	−	−	−
Salpiglossis sinuata	−	−	−	−	−	−

[1] Simple lytic ring.

[2] After 30 minutes two lytic rings appeared from both sides of the precipitin ring.

TABLE 3

Normal precipitin reaction of Solanum Dulcamara extract.

AGAINST NORMAL EXTRACTS OF	THE POTENCY OF THE PRECIPITIN REACTION AFTER					
	1 min.	5 min.	10 min	20 min.	30 min.	40 min.
Capsicum pyramidale	++	+++	++++	++++	+++	+++
Nicotiana rustica	−	−	−	−	−	−
Nicotiana glauca	—	—	—	—	—	—
Salpiglossis sinuata	+++	++++	++++	++++	+++	+++
Solanum Melongena	−	−	−	−	−	−
Solanum nigrum	−	−	−	−	−	−
Lycium Barbarum	+	+++	+++	+++	++	++
Datura Wrightii	++++	++++	++++	++++	+++	+++
Physalis peruviana	+++	++++	++++	++++	+++	+++

TABLE 4

Normal precipitin reaction of Salpiglossis sinuata extract.

AGAINST NORMAL EXTRACTS OF	THE POTENCY OF THE PRECIPITIN REACTION AFTER					
	1 min.	5 min.	10 min.	20 min.	30 min.	40 min.
Capsicum pyramidale[1]	−	−	−	−	−	−
Nicotiana rustica	+++	++++	++++	+++	+++	+++
Nicotiana glauca	+	++	+++	+++	++	++
Nicotiana Tabacum	+++	++++	++++	++++	+++	+++
Solanum nigrum	±	++	+++	+++	++	++
Solanum Melongena	+	+++	+++	+++	++	++
Solanum Dulcamara	+++	++++	++++	++++	+++	+++
Lycium Barbarum[1]	−	−	−	−	−	−
Datura Wrightii[1]	−	−	−	−	−	−
Solanum Lycopersicum	+	++	+++	+++	++	++
Physalis peruviana	−	−	−	−	−	−

[1] Simple lytic ring.

TABLE 5

Normal precipitin reaction of Lycium Barbarum extract.

AGAINST NORMAL EXTRACTS OF	THE POTENCY OF THE PRECIPITIN REACTION AFTER					
	1 min.	5 min.	10 min.	20 min.	30 min.	40 min.
Capsicum pyramidale	±	+	++	++	+	+
Nicotiana Tabacum	−	±	±	−	−	−
Nicotiana glauca	−	±	±	±	−	−
Nicotiana rustica	−	−	−	−	−	−
Solanum Melongena	−	±	−	−	−	−
Solanum nigrum[1]	−	−	−	−	−	−
Datura Wrightii	±	++	++	++	+	+
Salpiglossis sinuata[1]	−	−	−	−	−	−
Physalis peruviana	−	−	−	−	−	−
Solanum Dulcamara	+	+++	+++	+++	++	++
Solanum tuberosum	−	−	−	−	−	−
Nicotiana Rusbyi	−	−	−	−	−	−
Datura ferox	−	+	++	++	+	+

[1] Lytic ring.

TABLE 6

Normal precipitin reaction of Solanum Lycopersicum extract.

AGAINST NORMAL EXTRACTS OF	THE POTENCY OF THE PRECIPITIN REACTION AFTER					
	1 min.	5 min.	10 min.	20 min	30 min.	40 min.
Datura Wrightii	++++	++++	++++	++++	+++	+++
Capsicum pyramidale	−	±	+	+	+	±
Salpiglossis sinuata	+	++	+++	+++	++	++
Solanum Melongéna	−	−	−	−	−	−
Solanum tuberosum[1]	−	−	−	−	−	−
Nicotiana Tabacum	−	−	−	−	−	−
Nicotiana rustica	−	−	−	−	−	−
Lycium Barbarum	−	−	−	−	−	−
Datura ferox	±	+	+	+	±	±

[1] Simple lytic ring.

TABLE 7

Normal precipitin reaction of Capsicum pyramidale extract.

AGAINST NORMAL EXTRACTS OF	THE POTENCY OF THE PRECIPITIN REACTION AFTER					
	1 min.	5 min.	10 min.	20 min.	30 min.	40 min.
Datura Wrightii	−	±	±	±	−	−
Nicotiana Tabacum	−	−	−	−	−	−
Nicotiana glauca	−	−	±	−	−	−
Solanum Melongena	−	+	+	+	±	±
Lycium Barbarum	±	+	++	++	+	+
Salpiglossis sinuata[1]	−	−	−	−	−	−
Physalis peruviana[1]	−	−	−	−	−	−
Solanum tuberosum	−	−	−	−	−	−
Solanum nigrum	+	+++	++++	++++	+++	+++

[1] Simple lytic ring.

TABLE 8

Normal precipitin reaction of Nicotiana Rusbyi extract (plant B).

AGAINST NORMAL EXTRACTS OF	THE POTENCY OF THE PRECIPITIN REACTION AFTER					
	1 min.	5 min.	10 min.	20 min.	30 min.	40 min.
Nicotiana rustica	−	±	±	−	−	−
Nicotiana paniculata	−	−	−	−	−	−
Nicotiana glauca	−	−	−	−	−	−
Nicotiana Langsdorffii	±	++	+++	+++	++	++
Nicotiana alata	−	+	+	±	±	±
F_1 (*Nicotiana rustica* × *Nicotiana Tabacum*)	−	+	+	+	±	±
Nicotiana Tabacum	−	+	++	+	+	±
Nicotiana suaveolens	±	++	+++	+++	++	++
Petunia violacea	−	++	+++	+++	++	++

The best formation of the reaction rings in the normal precipitations occurs usually from 5 to 15 minutes after the beginning of the reaction. After 15 minutes, in most cases, the ring widens; and then both extracts begin to mix together, the upper layer penetrating into the lower one like small roots. Some particles of the floculation stick together, forming larger flocules which drop to the bottom; others obviously disappear, —that is, the precipitates dissolve again, the components in the tubes in most cases becoming gradually clearer and clearer after 15 to 20 minutes. In many cases when the precipitation is very weak, the precipitate completely disappears. In some combinations like *Solanum nigrum* + *Lycium Barbarum* (table 1), *Physalis peruviana* + *Capsicum pyramidale* (table 2) *Salpiglossis sinuata* + *Lycium Barbarum*, *Salpiglossis sinuata* + *Datura Wrightii*, *Salpiglossis sinuata* + *Capsicum pyramidale* (table 4) etc., between both layers a lytic (*clear*) ring appears, which is clearer than either extract. When the precipitation ring begins to spread above and below, it sometimes splits into two, with the formation of a lytic ring, appearing at about the same place that first the precipitation ring occurred. This is the phenomenon that gives the appearance of a double precipitation ring. The lytic ring which splits the precipitation ring in two is never as clear as the original component extracts, but clearer of course than the double precipitation ring. When the lytic phenomenon was observed, it usually followed after the precipitation phenomenon.

All reactions of the data reported in tables 1–8 were repeated at least 3 times, and where there was any doubt, 4–5 times. When different plants of the same species were used for the precipitin reaction, the results varied in some combinations within relatively narrow limits.

The greatest variation in the normal precipitin reaction, dependent

upon the individuality of the plants, showed itself in *Datura Wrightii*, *Solanum nigrum*, *Nicotiana Langsdorffii* and *Nicotiana Rusbyi*. Thus the normal precipitin reaction of plant B of *Nicotiani Rusbyi* (table 8) is not identical with the normal precipitin reaction of plant C (table 9).

TABLE 9

Normal precipitin reaction of Nicotiana Rusbyi extract (plant C).

AGAINST NORMAL EXTRACTS OF	THE POTENCY OF THE PRECIPITIN REACTION AFTER					
	1 min.	5 min.	10 min.	20 min.	30 min.	40 min.
Nicotiana rustica	−	+	+	±	±	−
Nicotiana paniculata	−	−	−	−	−	−
Nicotiana glauca	−	−	−	−	−	−
Nicotiana Langsdorffii	+	+++	+++	+++	++	++
Nicotiana alata	−	+	+	+	±	±
Nicotiana Tabacum	−	±	+	+	+	±
Nicotiana suaveolens	−	++	++	++	++	+
Petunia violacea	±	++	+++	+++	++	++

Some interrelation between the pH of the plant extracts and the normal precipitin reaction was expected. The pH was tested by Clark's (1920) color indicators. The results are presented in table 10. The determinations for the various species were obtained from plants growing under many different environmental conditions. In the table is given the average value of pH.

We can draw the conclusion from the data of table 10 in comparison with those of tables 1 to 9 that no correlation exists between normal precipitin reaction and hydrogen ion concentration.

ACQUIRED PRECIPITINS

By a series of experiments we have tried to answer the question: Is the plant cell (that is, the plant organism) able to acquire the capacity to produce precipitins; and, if so, how far is this ability specific? In order to answer this question, various plants were grafted together, and the physiological and morphological changes in the scions and stocks were studied.

Twenty shoots of *Nicotiana Rusbyi* were grafted on *Nicotiana rustica* the same afternoon. Both the shoots and the stock (*Nicotiana rustica*) plants were chosen as nearly alike as possible in age and size. In the successful grafting an approximately regular circulation of sap from the stock into the scion and *vice versa* begins usually from 3 to 4 days after the grafting was made. On the day when the graftings were made, extracts were obtained and the capacity for a normal precipitin reaction was

TABLE 10

Normal precipitin reaction—maximum titre and average value of pH. (0=*lytic ring*, ±=*trace*, +=+, ++=2, +++=3, ++++=4, *and*− = *no reaction*).

	AVERAGE VALUE OF PH.	*Capsicum pyramidale*	*Datura ferox*	*Datura Wrightii*	*Lycium Barbarum*	*Nicotiana alata*	*Nicotiana glauca*	*Nicotiana Langsdorffii*	*Nicotiana paniculata*	*Nicotiana Rusbyi*	*Nicotiana rustica*	F_1 (*N. rustica N. Tabacum*)	*Nicotiana Tabacum*	*Nicotiana suaveolens*	*Petunia violacea*	*Physalis peruviana*	*Salpiglossis sinuata*	*Solanum Dulcamara*	*Solanum Lycopersicum*	*Solanum Melongena*	*Solanum nigrum*	*Solanum tuberosum*
Capsicum pyramidale	5.8	−		±	2	+	±	2	+	±	+	0	+	0	−	0	0	4	+	2	4	−
Datura ferox	5.2		−		2	±													+			
Datura Wrightii	5.0	±		−	2								2				0	4	4	4	4	4
Lycium Barbarum	7.2	2	2	2	−	−	±	−	−	−	−	−	±	±	2	−	0	3	−	±	0	−
Nicotiana alata	5.6	+	±		−	−	−	−	−	+	−	−	−	−	−	−	−		−	−	−	2
Nicotiana glauca	5.6	±			±	−	−	−	−	−	−	−	−	−	−	2	3	−	−	−	+	−
Nicotiana Langsdorffii	5.4	2			−	−	−	−	−	3	−	−	−	−	+		−		−	−	−	2
Nicotiana paniculata	5.5	+			−	−	−	−	−	±	−	−	±	−	−		±		−	−	−	−
Nicotiana Rusbyi	5.6	±			−	+	−	3	±	−	+	+	2	3	3		−		+	+	3	−
Nicotiana rustica	5.2	+			−	−	−	−	−	+	−	−	−	−	−	3	4	−	−	−	2	±
F_1 (*N. rustica N. Tabacum*)	5.4	0			−	−	−	−	−	+	−	−	−	−	−		−		−	0	−	−
Nicotiana Tabacum	5.6	+	2		±	−	−	−	±	2	−	−	−	+	−		4		−	0	−	−
Nicotiana suaveolens	5.3	0			±	−	−	−	−	3	−	−	+	−	−		−		−	−	±	
Petunia violacea	5.1	−			2	−	−	+	−	3	−	−	−	−	−		−		−	−	−	
Physalis peruviana	7.6	0			−		2				3		−	−		−	−	4	−	−	2	−
Salpiglossis sinuata	7.7	0		0	0	−	3	−	±	−	4	−	4	−	−	−	−	4	3	3	3	
Solanum Dulcamara	6.0	4		4	3	−										4	4	−		−	−	
Solanum Lycopersicum	5.4	+	+	4	−	−	−		−	+	−	−	−	−	−		3		−	−	−	0
Solanum Melongena	5.7	2		4	±	−	−	−	−	+	−	0	0	−	−	−	3	−	−	−	−	
Solanum nigrum	6.4	4		4	0	−	+	−	−	3	2	−	−	±	−	2	3	−	−	−	−	
Solanum tuberosum	6.4	−		4	−	2	−	2	−	−	±	−	−			−			0			−

tested. On the 5th, 10th, 15th, 20th, 25th, 30th, 35th, 40th, 45th, 50th, 60th, 80th, and 100th days after the grafting, the acquired (immune) precipitin reaction and the normal precipitin reaction (if any) were determined. The difference between the precipitation capacity of the first and of the second (normal) test represents the acquired precipitation potency in the grafted union. In other words, the normal extract from *Nicotiana Rusbyi* and the normal extract from *Nicotiana rustica* were used as a control of immune (acquired) precipitins.

In table 11 is given for illustration a copy of the original notes from which the curves of figure 2 were drawn

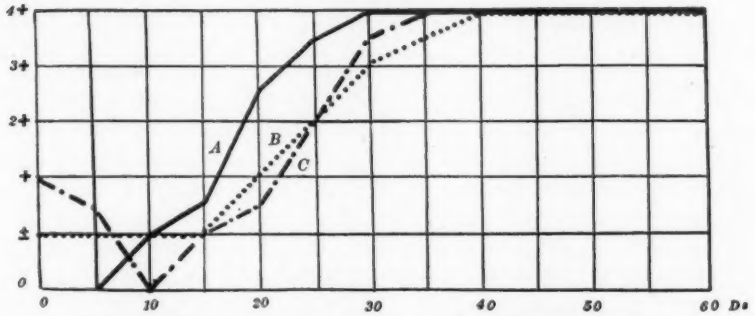

FIGURE 2.—Precipitin reaction capacity of shoots of *Nicotiana Rusbyi* plants A, B and C growing as scions on *Nicotiana rustica* stocks.

TABLE 11

Scion—Nicotiana Rusbyi (plant B)
Stock—Nicotiana rustica (N 28)
Grafting made on March 26, 1927
Test of the precipitin reaction—April 25, 1927
Ratio: 1 part of tissue extracted by 2 parts distilled water.

	PRECIPITIN REACTION WITH NORMAL EXTRACT OF NICOTIANA RUSTICA
Immune extract of *Nicotiana Rusbyi* against *Nicotiana rustica*	+++
Normal extract of *Nicotiana Rusbyi*	±

The results obtained in this way were summarized and plotted in figure 2. The curves show the precipitin reaction capacity of the shoots of *Nicotiana Rusbyi* plants A, B, and C growing as scions on *Nicotiana rustica*. When the grafts were made, the extract of plant A manifested no normal precipitin reaction "(−)", the extract of plant B a "trace", "(±)," and the extract of plant C showed "+" normal precipitin reaction

with *Nicotiana rustica* normal extract. At 10 days after grafting, the grafted shoots of plant A manifested a trace of precipitin reaction; and this reaction increased in intensity with the time of growth on *Nicotiana rustica* until the 30th day (++++). At later dates, the level of the precipitin reaction remained constant. The increase of the precipitin capacity of B scions began at the same time (10 days). It reached the highest potency, however, 40 days after grafting. The normal precipitin reaction "(+)" manifested at first by the extract of the shoots of plant C, decreased during the first 10 days. Later, it began to increase, and reached the highest point 35 days after grafting. The control tests which were always made when the immune precipitin capacity was tested, showed very slight variations from the control made at the time of grafting (compare the ordinate for 0 days, figure 2).

The curves on figure 2 shows very conspicuously the individual reaction of the several plants. The most striking immunological difference is between plant A and plant C.

A similar series of studies of the immune precipitin reactions was carried out in the subsequent combinations:

1. *Solanum tuberosum* grafted on *Nicotiana rustica.*
2. *Lycium Barbarum* grafted on *Solanum Lycopersicum.*
3. *Nicotiana Sanderae* grafted on *Datura ferox.*
4. *Nicotiana glauca* grafted on *Capsicum pyramidale.*

The highest precipitation capacity in the scion extracts was found to be 35–40–45 days after grafting. In tables 12, 13, 14, and 15 are given the highest immune potency of the four graft combinations mentioned above.

TABLE 12

Scion—Solanum tuberosum
Stock—Nicotiana rustica
Grafting made on March 23, 1927
Test of the precipitin reaction—May 2, 1927
Ratio: 1 part of tissue extracted by 2 parts distilled water.

	PRECIPITIN REACTION WITH NORMAL EXTRACT OF NICOTIANA RUSTICA
Immune extract of *Solanum tuberosum* against *Nicotiana rustica*	+++
Normal extract of *Solanum tuberosum*	±

TABLE 13

Scion—Lycium Barbarum
Stock—Solanum Lycopersicum
Grafting made on March 25, 1927

Test of the precipitin reaction—April 30, 1927
Ratio: 1 part of tissue extracted by two parts distilled water.

	PRECIPITIN REACTION WITH NORMAL EXTRACT OF SOLANUM LYCOPERSICUM
Immune extract of *Lycium Barbarum* against *Solanum Lycopersicum*	++
Normal extract of *Lycium Barbarum*	−

TABLE 14

Scion—Nicotiana Sanderae
Stock—Datura ferox
Grafting made on April 15, 1927
Test of the precipitin reaction—May 29, 1927
Ratio: 1 part of tissue extracted by two parts distilled water.

	PRECIPITIN REACTION WITH NORMAL EXTRACT OF DATURA FEROX
Immune extract of *Nicotiana Sanderae* against *Datura ferox*	++
Normal extract of *Nicotiana Sanderae*	±

TABLE 15

Scion—Nicotiana glauca
Stock—Capsicum pyramidale
Grafting made on February 10, 1927
Test of the precipitin reaction—March 17, 1927
Ratio: 1 part of tissue extracted by two parts distilled water.

	PRECIPITIN REACTION WITH NORMAL EXTRACT OF CAPSICUM PYRAMIDALE
Immune extract of *Nicotiana glauca* against *Capsicum pyramidale*	++
Normal extract of *Nicotiana glauca*	±

Very slight acquirement of precipitins was found in the following graft unions:

1. *Nicotiana Rusbyi* scion on *Nicotiana glauca.*
2. *Solanum Lycopersicum* scion on *Datura ferox.*
3. *Nicotiana Langsdorffii* scion on *Nicotiana rustica.*
4. F_1 (*Nicotiana Tabacum*×*Nicotiana Rusbyi*) scion on *Nicotiana Sanderae*
5. *Lycium Barbarum* scion on *Datura ferox.*
6. *Datura Wrightii* scion on *Nicotiana Tabacum.*
7. *Lycium Barbarum* scion on *Nicotiana Tabacum.*

No acquirement of precipitins was found in the following graft unions:

1. *Nicotiana Tabacum* scion on *Nicotiana rustica.*

2. *Nicotiana rustica* scion on *Nicotiana quadrivalvis.*
3. *Nicotiana Tabacum* scion on *Solanum nigrum.*
4. *Salpiglossis sinuata* scion on *Datura ferox.*
5. *Nicotiana glauca* scion on *Nicotiana rustica.*
6. *Solanum Melongena* scion on *Solanum nigrum.*
7. *Datura Wrightii* scion on *Solanum nigrum.*
8. *Nicotiana Tabacum* scion on *Solanum Nelongena.*
9. *Solanum nigrum* scion on *Nicotiana Sanderae.*
10. *Nicotiana Tabacum* scion on *Solanum tuberosum.*
11. *Nicotiana Tabacum* scion on *Nicotiana Langsdorffii.*

A decrease of the normal precipitins was observed in the following unions:

1. *Solanum tuberosum* grafted on *Datura Wrightii.*
2. *Solanum Melongena* grafted on *Datura Wrightii.*
3. *Solanum Lycopersicum* grafted on *Datura Wrightii.*
4. *Nicotiana Tabacum* grafted on *Datura Wrightii.*

The gradual decrease of the normal precipitin reaction in *Solanum tuberosum* (T), *Solanum Melongena* (M), and *Solanum Lycopersicum,*(L) when grafted on *Datura Wrightii,* are presented in figure 3.

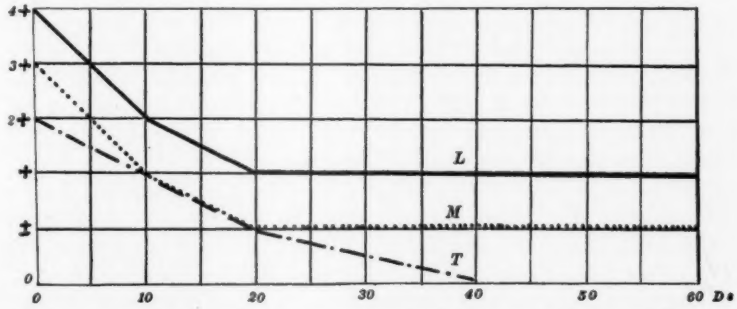

FIGURE 3.—Gradual decrease of the normal precipitins in shoots of *Solanum tuberosum* (T), *Solanum Melogena* (M) and *Solanum Lycopersicum* (L) grafted on *Datura Wrightii.*

A lytic ring was obtained from the union: *Solanum tuberosum* grafted on *Nicotiana alata* 20 days after the grafting.

A general question of importance to serology is the precipitin capacity of gradually diluted serum. It also has significance in plant immunology, because the precipitin capacity can be measured in this way. The highest limits of dilution of immune extracts giving precipitin reaction are shown

on table 16 and 17, and in figure 4. The graft union is *Nicotiana Rusbyi* scion on *Nicotiana rustica*.

TABLE 16

Precipitin reaction between the immune extract of Nicotiana Rusbyi grafted on Nicotiana rustica and antigenic extract of Nicotiana rustica. (Diluent—distilled water)

IMMUNE EXTRACT DILUTED	PRECIPITIN REACTION AFTER					
	1 min.	5 min.	10 min.	20 min.	30 min.	40 min.
1:0	++++	++++	++++	++++	++++	++++
1:1	+++	++++	++++	++++	++++	++++
1:2	+++	++++	++++	++++	++++	++++
1:4	+++	+++	+++	++++	++++	++++
1:8	+++	+++	+++	+++	++++	++++
1:12	++	+++	+++	+++	+++	+++
1:16	++	++	+++	+++	+++	+++
1:20	+	++	++	++	++	++
1:24	+	+	++	++	++	++
1:30	±	+	+	++	++	++
1:36	±	±	+	+	+	+
1:50	−	±	+	+	+	+
1:75	−	−	±	+	+	+
1:100	−	−	−	±	±	±
1:200	−	−	−	−	−	−

TABLE 17

Precipitin reaction between immune extract of Nicotiana Rusbyi grafted on Nicotiana rustica and antigenic extract of Nicotiana rustica. (Diluent—distilled water)

ANTIGENIC EXTRACT DILUTED	PRECIPITIN REACTION AFTER					
	1 min.	5 min.	10 min.	20 min.	30 min.	40 min.
1:0	++++	++++	++++	++++	++++	++++
1:8	+++	++++	++++	++++	++++	++++
1:16	++	+++	+++	++++	++++	++++
1:32	+	+	++	+++	+++	+++
1:64	±	±	+	++	++	++
1:128	−	−	±	+	+	+
1:256	−	−	−	−	−	−

Similar results were obtained from *Nicotiana glauca* grafted on *Capsicum pyramidale* and from *Solanum tuberosum* grafted on F_1 (*Nicotiana rustical* var. *humilis*×*Nicotiana rustica* var. *brasilia*).

From a comparison of table 16 with table 17, it appears that the precipitin reaction drops down more quickly when the immune extract is diluted.

The limit of dilution of the immune extract shown in table 16 and in figure 4 is 1:100—not nearly as high a dilution as can be reached with

rabbit serum. But we cannot make a comparison between the rabbit serum and the plant extract. The animal cell (that is organism) is more sensitive and reactable (MÜLLER 1926) and disposes of more antibody formative materials than the plant cell. Moreover, the basic amount of extract (normal dilution) we usually used represents an extraction of 1 part plant organs (leaves and stems) to 2 parts of distilled water. That is relatively a very high dilution.

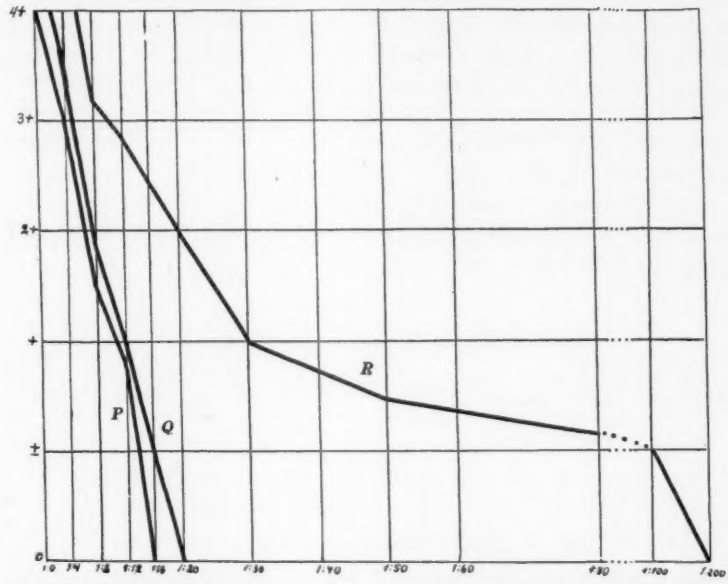

FIGURE 4.—Precipitation capacity of *Nicotiana Rusbyi* immune extracts against *Nicotiana rustica* normal extracts. Curve P-reaction of scions extracted immediately after cutting. Curve Q-reaction of scions extracted 4 days after cutting. Curve R-reaction of shoots extracted 2 days after-cutting.

The Uhlenhuth's ring observed between an immune and an antigenic extract keeps its form a longer time (table 16 and 17) than in a normal precipitin reaction, and the immune-antigenic extract components mix more slowly.

The immune extracts used in obtaining the data for tables 16 and 17 and curve R of figure 4 were produced from shoots growing as grafts 40 days and kept in a glass of water 2 days after cutting.

Curve R (figure 4) is drawn from the data of 3 tests, and includes the

results from table 16. Curve Q (figure 4) shows the precipitin capacity of two shoots grown as grafts 40 days, and kept 4 days in a glass of water after cutting. Curve P represents the precipitin potency of 4 scions of *Nicotiana Rusbyi* grown 40 days in graft union with *Nicotiana rustica* stocks, as were those of curves R and Q, but extracted immediately after cutting.

Comparing the curves in figure 4, one may conclude that the shoots extracted two days after cutting (curve R) have a higher precipitin potency than the shoots of the other two cases. Probably a part of the precipitin in the shoots extracted immediately after cutting (curve P) is neutralized by the antigenic sap coming up from the stock. The drop of the curve Q probably is due to the failure of antigenic agents over a relatively longer time.

When shoots of different ages were compared, the greatest amount of precipitins was found in the leaves and stems of about 20 to 70 days old. Both younger and older tissues contain less precipitins.

The results obtained from the material treated by tartaric acid-alcohol-ether, ether, chloroform and chloroform-alcohol before the extraction compared with the material not treated are as follows:

TABLE 18[1]

a. Material treated by tartaric-acid-alcohol-ether 2 hrs.

Immune extract of *Nicotiana Sanderae*	+	Antigenic extract of *Datura ferox*	=+
Normal extract of *Nicotiana Sanderae*	+	Normal (antigenic) extract of Datura *ferox*	=−

b. Non-treated material.

Immune extract of *Nicotiana Sanderae*	+	Antigenic extract of *Datura ferox*	=++
Normal extract of *Nicotiana Sanderae*	+	Normal extract of *Datura ferox*	=±

c. Material treated by ether three days.

Immune extract of F_1 (*Nicotiana Tabacum* × *Nicotiana Rusbyi*)	+	Antigenic extract of *Nicotiana Sanderae*	=±
Normal extract of F_1 *Nicotiana Tabacum* × *Nicotiana Rusbyi*	+	Normal extract of *Nicotiana Sanderae*	=−

d. Non-treated material.

Immune extract of F_1 (*Nicotiana Tabacum* × *Nicotiana Rusbyi*)	+	Antigenic extract of *Nicotiana Sanderae*	=+++
Normal extract of F_1 (*Nicotiana Tabacum* × *Nicotiana Rusbyi*)	+	Normal extract of *Nicotiana Sanderae*	=+

e. Material treated by ether 2 hours.

Immune extract of *Nicotiana Rusbyi*	+	Antigenic extract of *Nicotiana rustica*	=+to++
Normal extract of *Nicotiana Rusbyi*	+	Normal extract of *Nicotiana rustica*	=−

f. Non-treated material.

Immune extract of *Nicotiana Rusbyi*	+ Antigenic extract of *Nicotiana rustica*	=++++
Normal extract of *Nicotiana Rusbyi*	+ Normal extract of *Nicotiana rustica*	=+

g. Material treated 2 hours with chloroform-alcohol.

Immune extract of *Nicotiana Rusbyi*	+ Antigenic extract of *Nicotiana rustica*	=+++
Normal extract of *Nicotiana Rusbyi*	+ Normal extract of *Nicotiana rustica*	=+

h. Material treated 2 hours with chloroform.

Immune extract of *Nicotiana Rusbyi*	+ Antigenic extract of *Nicotiana rustica*	=+++
Normal extract of *Nicotiana Rusbyi*	+ Normal extract of *Nicotiana rustica*	=+

i. Material treated 2 hours with chloroform.

Immune extract of *Nicotiana Sanderae*	+ Antigenic extract of *Datura ferox*	=++
Normal extract of *Nicotiana Sanderae*	+ Normal extract of *Datura ferox*	=+

[1] In all series of experiments in table 18, 1 part of tissue was extracted by 1 part distilled water

Parallel experiments for observing the precipitation capacity for 24 hours (MEZ) with the components of a, c, e, g, and h in ratio 1:1 were also carried out. The results did not differ from those obtained in 40 minutes.

The most marked decrease of the precipitin capacity was found in the material treated with ether before the water extraction was made.

The normal extract of *Nicotiana Rusbyi* in one case manifested the same precipitin potency (+++) as the immune extract when tested against the antigenic extract of *Nicotiana rustica* in undiluted form. After dilution of the extracts, however, the precipitin potency of the normal extract decreased faster than that of the immune extract; and the Uhlenhuth ring kept its form and the precipitate its density longer in the case of the immune extract than in the normal extract.

Optimum growing temperatures (25°–30°C) and intense light increased the precipitin content.

The data reported in the preceding pages give us an answer to our first question. *Plants,—that is, the cells in the plants,—are able to produce antibodies.* This statement will be confirmed later by other observations of a different nature.

Experiments were made in which it was proposed to test the specific reactivity of the immune extracts. The data obtained are given in tables 19, 20, 21, and 22.

TABLE 19

Acquired precipitin reactions of Nicotiana Rusbyi immune extract (Nicotiana Rusbyi grafted on Nicotiana rustica).

AGAINST NORMAL EXTRACT OF	PRECIPITIN REACTION AFTER					
	1 min.	5 min.	10 min	20 min.	20 min.	40 min.
Nicotiana rustica	++++	++++	++++	++++	++++	++++
Nicotiana paniculata	++	+++	+++	+++	+++	+++
Nicotiana glauca	+	++	+++	+++	+++	+++
F_1 (*Nicotiana rustica* × *Nicotiana Tabacum*)	±	+	++	++	++	++
Nicotiana Tabacum	−	±	+	+	+	+
Nicotiana alata	++	+++	++++	++++	++++	+++
Nicotiana Langsdorffii	+++	++++	++++	++++	++++	++++

TABLE 20

Acquired precipitin reactions of Nicotiana Rusbyi immune extract of table 19 diluted in distilled water 1:4.

AGAINST NORMAL EXTRACT OF	PRECIPITIN REACTION AFTER					
	1 min.	5 min.	10 min.	20 min.	30 min.	40 min.
Nicotiana rustica	+++	++++	++++	++++	++++	++++
Nicotiana paniculata	±	+	++	++	++	++
Nicotiana glauca	−	±	+	+	+	+
F_1 (*Nicotiana rustica* × *Nicotiana Tabacum*)	−	−	−	−	−	−
Nicotiana Tabacum	−	−	−	−	−	−
Nicotiana alata	+	++	+++	+++	++	++
Nicotiana Langsdorffii	++	+++	+++	+++	+++	++

TABLE 21

Acquired precipitin reactions of Nicotiana Rusbyi immune extract of table 19 diluted in distilled water 1:8.

AGAINST NORMAL EXTRACT OF	PRECIPITIN REACTION AFTER					
	1 min.	5 min.	10 min	20 min.	30 min.	40 min.
Nicotiana rustica	++	+++	+++	+++	+++	+++
Nicotiana paniculata	±	+	+	+	+	+
Nicotiana glauca	−	−	±	±	±	±
F_1 (*Nicotiana rustica* × *Nicotiana Tabacum*)	−	−	−	−	−	−
Nicotiana Tabacum	−	−	−	−	−	−
Nicotiana alata	±	+	+	+	+	±
Nicotiana Langsdorffii	±	+	++	++	++	+

TABLE 22

Acquired precipitin reactions of Nicotiana Rusbyi immune extract of table 19 diluted in distilled water 1:16.

AGAINST NORMAL EXTRACT OF	PRECIPITIN REACTION AFTER					
	1 min.	5 min.	10 min.	20 min.	20 min.	40 min.
Nicotiana rustica	+	++	++	++	++	++
Nicotiana paniculata	−	−	−	−	−	−
Nicotiana glauca	−	−	−	−	−	−
F_1 (*Nicotiana rustica* × *Nicotiana Tabacum*)	−	−	−	−	−	−
Nicotiana Tabacum	−	−	−	−	−	−
Nicotiana alata	−	−	±	±	−	−
Nicotiana Langsdorffii	−	±	+	+	±	−

Nicotiana Rusbyi extract immunized against *Nicotiana rustica* by grafting always gave the heaviest precipitate when tested against its antigenic extract, namely the normal extract of *Nicotiana rustica*. Then follow in order the extracts of *Nicotiana paniculata*, *Nicotiana glauca*, F_1 (*Nicotiana rustica* × *Nicotiana Tabacum*) and *Nicotiana Tabacum*. Three species *Nicotiana rustica*, *Nicotiana paniculata*, and *Nicotiana glauca* belong to the subgenus or section Rustica; the species *Nicotiana Tabacum* and *Nicotiana Rusbyi* belong to the section Tabacum (Comes). The hybrid *Nicotiana rustica* × *Nicotiana paniculata* is partially fertile, (EAST 1921), while the hybrid *Nicotiana rustica* × *Nicotiana glauca* produces embryos which are viable only 10–15 days. The genetic compatibility between *Nicotiana rustica* and *Nicotiana paniculata* and between *Nicotiana rustica* and *Nicotiana glauca* thus corresponds with the precipitin reaction obtained. *Nicotiana rustica* × *Nicotiana Tabacum* also produces a partially fertile F_1 generation; (in back crosses but since *Nicotiana Tabacum* is closely related to *Nicotiana Rusbyi*, the lower precipitin reaction is to be expected. The F_1 hybrid *Nicotiana rustica* × *Nicotiana Tabacum* usually gives a precipitin reaction between those of *Nicotiana rustica* and *Nicotiana Tabacum*. The powerful precipitation with *Nicotiana Langsdorffii* extract and with *Nicotiana alata* extract is due chiefly to the normal precipitins (compare with tables 8 and 9); nevertheless, heterogenetic (nonspecific) precipitins are also present.

In one case a high precipitin reaction was obtained when *Nicotiana Rusbyi* immune (against Nicotiana rustica) extract was tested against *Nicotiana rustica* and against *Nicotiana Tabacum* normal extracts (ex-

tracts undiluted – reaction + + +). For the elimination of the normal precipitins an experiment was performed as follows:

A. 2cc. *Nicotiana Tabacum* normal extract was added to 2cc. *Nicotiana Rusbyi* normal extract (Tube A).

B. 2cc. *Nicotiana rustica* normal extract was added to 2cc. *Nicotiana Rusbyi* normal extract (Tube B).

After 40 minutes A and B components were filtered separately. The filtrates gave the following results with *Nicotiana Rusbyi* immune extract:

1. A-filtrate + *Nicotiana Rusbyi* immune extract = –
2. B-filtrate + *Nicotiana Rusbyi* immune extract = + +

The reaction potency in the second combination (+ +) remained about the same even when 3cc. instead of 2cc. of *Nicotiana Rusbyi* normal extract were added to B.

This experiment shows that the precipitation between *Nicotiana Tabacum* normal extract and *Nicotiana Rusbyi* extract (immune against *Nicotiana rustica*) is simply a normal precipitation and not a reaction indicating acquired immunity.

A reaction similar to that shown in table 21 was obtained by an immune extract from a tuber formed in the air on a scion of *Solanum tuberosum* grafted on *Nicotiana rustica* [F_1 (*Nicotiana rustica* var, *humilis* × *Nicotiana rustica* var. *texana*)]. The results are shown in table 23.

TABLE 23

Acquired precipitin reactions of Solanum tuberosum (tuber) extract (Solanum tuberosum grafted on Nicotiana rustica 45 days).

NORMAL EXTRACT OF	PRECIPITIN REACTION AFTER					
	1 min.	5 min.	10 min.	20 min.	30 min.	40 min.
Nicotiana rustica	+	+ +	+ + +	+ + +	+ + +	+ + +
Nicotiana paniculata	–	±	+	+	+	+
Nicotiana glauca	–	–	–	–	–	–
Nicotiana Tabacum	–	–	–	–	–	–
F_1 (*Nicotiana rustica* × *Nicotiana Tabacum*)	–	–	–	–	–	–
Nicotiana Rusbyi	–	–	–	–	–	–
Nicotiana alata	±	+	+ +	+ +	+ +	+
Nicotiana Langsdorffii	+	+ + +	+ + +	+ + +	+ +	+ +

In table 24 the normal precipitin reactions of normal extract of Solanum (tuber) are given for comparison.

TABLE 24

Normal precipitin reactions of Solanum tuberosum (tuber) normal extract.

NORMAL EXTRACT OF	PRECIPITIN REACTION AFTER					
	1 min.	5 min.	10 min	20 min.	20 min.	40 min.
Nicotiana rustica	–	–	–	–	–	–
Nicotiana paniculata	–	–	–	–	–	–
Nicotiana glauca	–	–	–	–	–	–
Nicotiana Tabacum	–	–	–	–	–	–
F_1 (*Nicotiana rustica* × *Nicotiana Tabacum*)	–	–	–	–	–	–
Nicotiana Rusbyi	–	–	–	–	–	–
Nicotiana alata	±	+	++	++	+	+
Nicotiana Langsdorffii	+	++	++	++	++	+

It is obvious that a great deal of the precipitation with *Nicotiana alata* and *Nicotiana Langsdorffii* (table 23), if not all, is due to normal precipitins; while the reaction with *Nicotiana rustica* and *Nicotiana paniculata* is evidently acquired.

Another demonstration of specific precipitin reaction is presented graphically in figure 5.

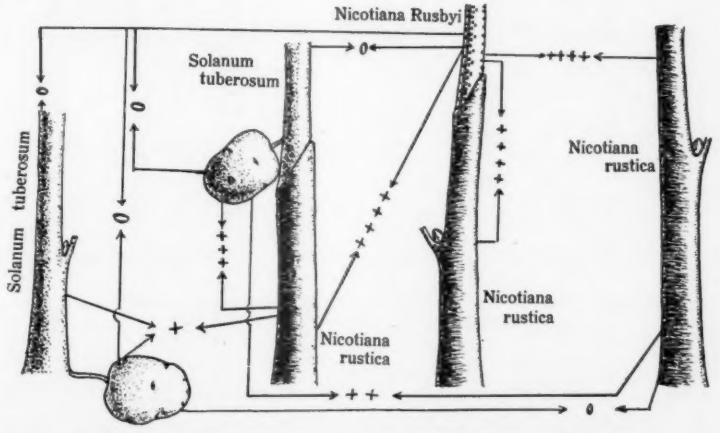

FIGURE 5.—Scheme for showing acquired precipitin reaction after grafting.

THE BEHAVIOR OF THE PLANT PRECIPITIN AND ANTIGEN TO SOME PHYSICAL AND CHEMICAL AGENTS

The behavior of antigen and precipitin to various agents in animals has been studied by many investigators. Although the data exhibits many

discrepancies, they appear to support the statement that high temperature diminishes the precipitin potency both of the immune serums and of the antigens.

In order to determine the influence of high temperatures on the immune and the antigenic extracts, the following experiments were carried out:

Immune and antigenic extracts in separate glass tubes were kept in water baths at 25°C, 60°C, 80°C, and 100°C respectively, for 30 minutes; after which, the precipitin potencies of the extracts were tested. Curves

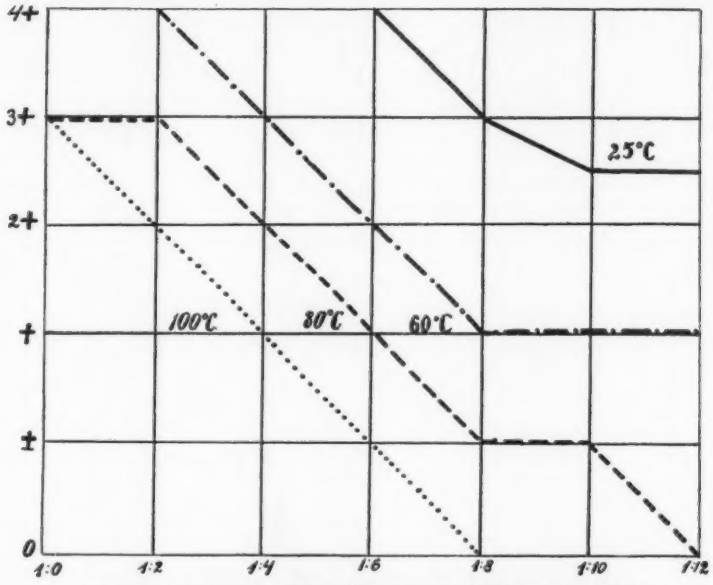

FIGURE 6.—Decrease of precipitation capacity of the immune extract in 30 minutes at 60°C , 80°C and 100°C temperature.

from the data obtained from the immune extract activity of *Nicotiana Rusbyi* against *Nicotiana rustica* antigen are plotted in figure 6. The curves of normal antigenic *Nicotiana rustica* extracts are plotted infigure 7. The precipitin reaction decreases with the increase of temperature. When the immune extract is treated in the same way, its precipitin reaction with the antigenic extract decreases more strikingly than when the antigenic extracts are so treated.

It was found that filter paper absorbs the precipitins and the antigens, thus causing the reaction potency to diminish.

The influence of formaldehyde, toluol, chloroform, H-ion, OH-ion, and certain metal-ions, as Pb-, Fe-, and Hg- on the antigen has been studied by OBERMEYER and PICK (1906) and by PICK (1912). These authors stated that all such agents make a change in the protein molecule similar to that produced by high temperature. LANDSTEINER and LAMPL (1917) did not confirm PICK's conclusion concerning the activity of formaldehyde on the antigen, but BAIVY (1926) found that. . . . "par l'action du

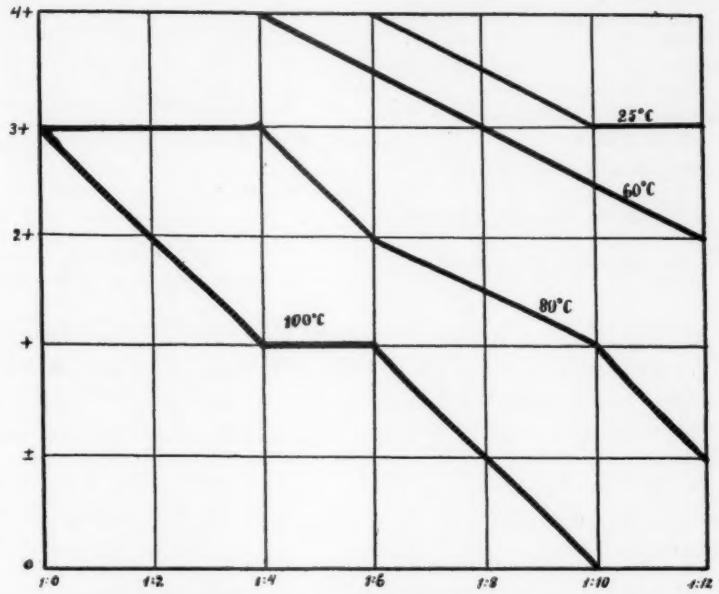

FIGURE 7.—Decrease of precipitation capacity of the antigenic extract in 30 minutes at 60°C, 80°C and 100°C temperature.

formol la précipitin perd immédiatement et complètement son activité; la proportion suffisant de formol étant, dans certains cas, de 1 per 1000." ABDERHALDEN (1922), moreover, found a decrease of the "plasma" and "sera" reaction capacity when treated by phenol, totuol and chloroform in so-called Abderhalden's reaction.

The reaction capacity of the immune extract of *Nicotiana Rusbyi*, treated by various chemical agents, against the *Nicotiana rustica* extract is shown in table 25.

TABLE 25

Reaction of treated immune extract of Nicotiana Rusbyi against normal Nicotiana rustica extract.

Immune extract	+	Antigenic extract	=++++
Immune extract saturated with $MgSO_4$ and filtered	+	Antigenic extract	=−
Immune extract saturated with Na_2SO_4 and filtered	+	Antigenic extract	=−
Immune extract saturated with $(NH_4)_2SO_4$ and filtered	+	Antigenic extract	=++++
Immune extract saturated with $Li_2Cr_2O_7$ and filtered	+	Antigenic extract	=−
(Immune extract+formaldehyde (40 per cent) in ratio 20:1) after 4 hours	+	Antigenic extract	=+++
(Immune extract+ chloroform in ratio 1:1) after 2 days	+	Antigenic extract	=+++
(Immune extract+toluol in ratio 1:1) after 2 days	+	Antigenic extract	=+++
(1 cc. Immune extract+1 drop 10 per cent CH_3COOH)	+	Antigenic extract	=++++
(1 cc. Immune extract+1 drop 10 per cent KOH)	+	Antigenic extract	=++++

In 1922, MASON pointed out that precipitin reactions are obtainable within a pH range of 4.5 to 9.5, and the precipitation is no heavier at the iso-electric point either of the serum or of the antigen; if the pH is outside of this range, no precipitate forms, and an existing precipitate will be redissolved by such H-ion or OH-ion concentrations.

In the present study, the hydrogen ion concentration has no marked influence on the precipitin reaction. The data for this conclusion are given in table 26.

TABLE 26

Immune extract diluted 1:10 (pH−5.6)	+	Antigenic extract (pH=5.4)	=+to++
(Immune extract diluted 1:10+HCl) with pH=1.8	+	Antigenic extract (pH=5.4)	=++
(Immune extract diluted 1:10+NaOH) with pH=9.8	+	Antigenicextract (pH=5.4)	=++
Immune extract diluted 1:10 (pH=5.6)	+	(Antigenic extract+HCl) with pH=3.0	=+
Immune extract diluted 1:10 (pH=5.6)	+	(Antigenic extract+NaOH) with pH=8.4	=+

The hydrogen ion concentration in the scion is usually altered to a condition nearly intermediate between that of scion plant and stock plant (table 27).

TABLE 27

pH determined by Clark's color indicators (compare table 10).

EXTRACT OF	pH
Lycium Barbarum	7.2
Lycium Barbarum scion on Solanum Lycopersicum	6.8
Solanum Lycopersicum	5.4
Nicotiana Tabacum	5.6
Lycium Barbarum scion on Nicotiana Tabacum	5.8
Nicotiana Tabacum scion on Solanum Melongena	5.6–5.8
Solanum Melongena	5.6–5.8
Solanum Melongena scion on Datura Wrightii	5.6
Datura Wrightii	5.0
Solanum tuberosum scion on Datura Wrightii	5.8–6.0
Solanum tuberosum	6.4
Solanum Lycopersicum scion on Datura Wrightii	5.2–5.4
Solanum tuberosum scion on Nicotiana rustica	6.0–6.2
Solanum tuberosum scion on Nicotiana Tabacum	6.0–6.2
Nicotiana rustica	5.2
Nicotiana rustica scion on Nicotiana quadrivalvis	4.8
Nicotiana quadrivalvis	4.4–4.6
Nicotiana Rusbyi scion on Nicotiana rustica	5.4–5.6
Nicotiana Rusbyi	5.6
Nicotiana Rusbyi scion on Nicotiana glauca	5.6
Nicotiana glauca	5.6
Nicotiana glauca scion on Capsicum pyramidale	5.6
Capsicum pyramidale	5.8
Datura Wrightii scion on Nicotiana Tabacum	5.2
Nicotiana Tabacum scion on Datura Wrightii	5.4
Solanum nigrum scion on Nicotiana Tabacum	5.6–5.8
Solanum nigrum	6.4
Nicotiana Tabacum scion on Solanum nigrum	5.6–5.8
Solanum Melongena scion on Solanum nigrum	5.8
Datura ferox scion on Solanum nigrum	5.2–5.4
Datura ferox	5.2
Lycium Barbarum scion on Datura ferox	5.6

A few years ago it was thought that all of the different antigens (sera, red blood corpuscles, egg albumine, milk, etc.) belonged to one chemical group of substances, namely to the proteins. "No antigen effect without protein" was a thesis generally accepted. Recently, the view that the proteins are the only antigens has been definitely changed. New evidence has indicated an antigenic nature for certain non-proteid substances, namely lipoids, bacterial distillates, carbohydrates, and alkaloids.

In 1909, PICK and SCHWARZ suggested on the antigenic property of the lipoids. MEYER (1910–1912) pointed out that for the antigenic activity, it is necessary to have other additional agents (probably proteins) whose

rôle is "Ictus immunisatorius" (EHRLICH und MORGENROTH's term). His statement was received with skepticism. Later (1912–1914) he obtained antisera by lipoid-antigens which reacted with lipoid antigens but not with those of proteins.

In 1911 FORSSMANN has found that emulsion of guinea pig kidney, injected into rabbits, induces antibody formation against sheep blood cells. It was established that this so-called heterogenetic antigen is not a protein but a lipoid, soluble in hot alcohol.

GAY and ROBERTSON (1912) denied the antigenic effect both of protamines and also of protamines in combination with caseinogen. GAMALEIA (1927) claimed that distillate products of the group of protamin substances and alkaloids can also cause immunity. YERMOLJEWA (1927) made similar statements. She obtained immune sera which gave the positive "complement deviation reaction" and the "Pfeiffer-phenomenon" with distillates of bacteria. Finally we must mention HEILDERBERGER, GOEBEL and AVERY recent investigations (1926). They have isolated I,II and III Type substances of Pneumococcus. Substance Type III is a "nitrogen-free polysaccharide" and "in as high a dilution as 1:6,000,000 it still reacts with Type III anti-pneumococcus serum."

Nevertheless, many serologists up to the present time are very skeptical about the antigenic effect of the substances outside of the protein group.

About the nature of the antigens and antibodies in the plants one can say very little. It is impossible to draw definite conclusions from the observed interaction between scion and stock or from the behavior of the antigenic and immune extracts to various physical and chemical agents reported above.

OBSERVED PHENOMENA EXPLICABLE BY ANTIGEN-ANTIBODY REACTION IN VIVO

In almost all intergeneric graft unions it was necessary for a satisfactory growth of the scion to maintain an approximate equality between the amount of green mass (leaves and stems) of the scion and the stock in the unions. As soon as the scion predominates, it either kills the stock or injures it so that no more growth is manifested. This occurred most frequently when the stock predominated. This phenomenon was not observed very frequently, however, in the graft unions between species.

The grafts of *Nicotiana glauca* on *Capsicum pyramidale* (see table 15) grew relatively well 4–5 weeks after grafting and sometimes gave shoots 25 cm long, of which few produced even flowers. After 4–5 weeks, the

growth of the scion shoots stopped and the scions began to die. On *Capsicum pyramidale* stock on which 5 scions of *Nicotiana glauca* had died, further attempts were made to graft new scions of the same species as before, but none of these scions grew. Since the inhibition of growth of the scion shoots approximately 4-5 weeks after the first grafting, coincides with the period of greatest antibody production, the failure of subsequent grafts on that stock which had already killed their scions is undoubtedly due to the antibodies induced in the stock by the first series of graftings.

FIGURE 8.—*Nicotiana Tabacum* var. *macrophylla*. G-shoot grafted on *Datura Wrightii*. N-non grafted shoot.

When *Nicotiana Tabacum* var. *macrophylla* is grafted on *Datura Wrightii*, the scions grow relatively normally, especially if equilibrium between the mass of the scion and the stock is retained. The flowers, however, could not develop normal corollas, and the top of the calyx leaves was usually more or less destroyed (figure 8). In periods of intense sunshine and unsatisfactory water supply, all corollas, as well as the greater part of the calyx leaves are shrunken and destroyed. In cloudy weather, when there was an adquate water supply for 10 or 12 days, the scions developed almost normal, but not completely normal, flowers.

The highest degree of disturbance in corolla and calyx is accompanied by a disturbance in the cell division of the pollen mother cells. The nongrafted *Nicotiana Tabacum macrophylla* plants growing around the grafted

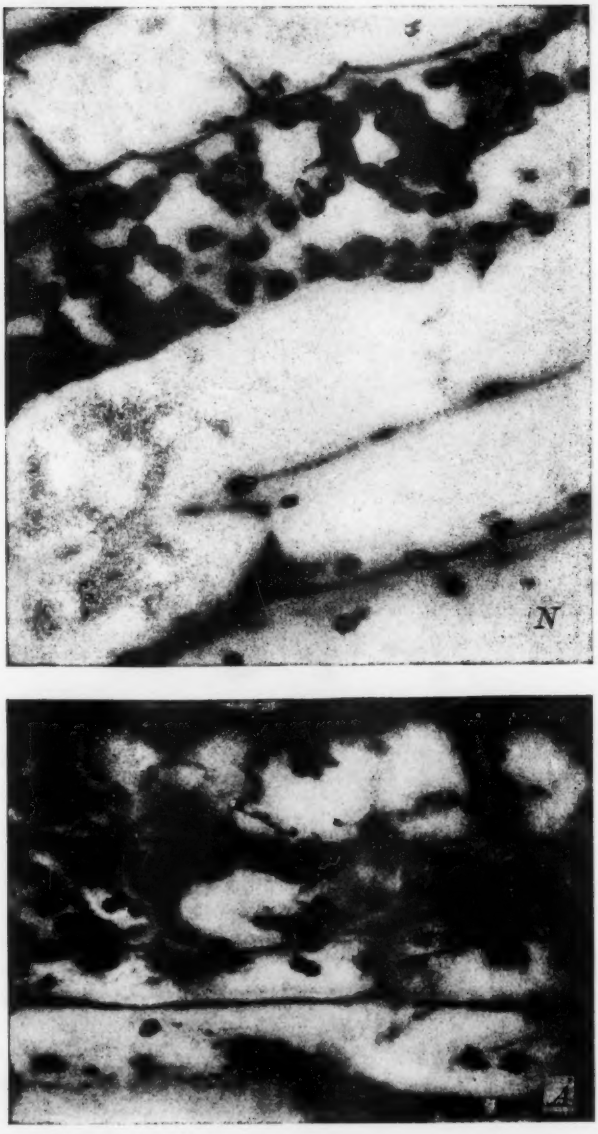

FIGURE 9.—N—normal chloroplasts of *Nicotiana glauca* grafted on *Capsicum pyramidale* from adistance of 2 cm from the callus. A—agglutinated chloroplasts of the same graft union in the tissue near to the callus.

ones in the same environmental conditions always showed normal cell division in the pollen mother cells and normally developed pollen grains;

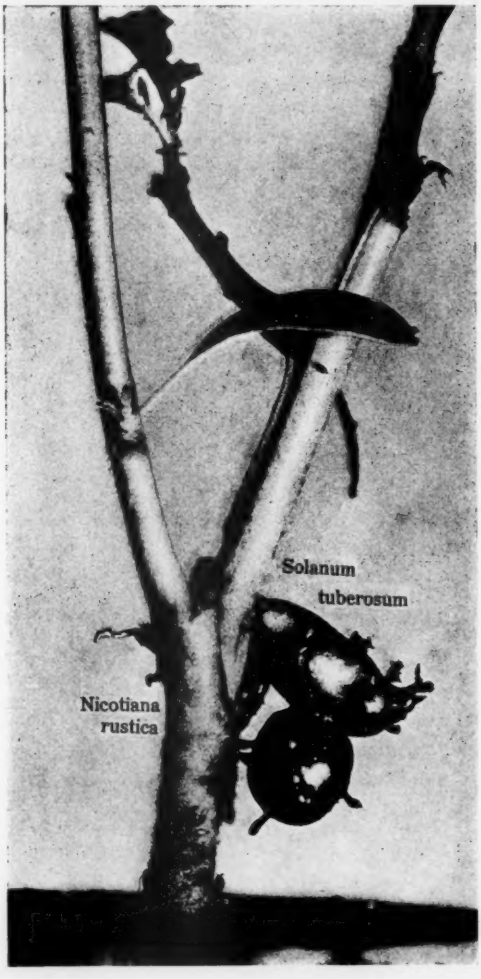

FIGURE 10.—*Solanum tuberosum* grafted on *Nicotiana* rustica.

while the shoots grafted on *Datura Wrightii* showed about 25–30 percent of abortive pollen grains, the corolla being entirely destroyed. The appear-

ance of abortive pollen grains is due to the abnormality in the reduction cell division in the pollen mother cells.

Abnormal heterotypical and homeotypical cell division of the pollen mother cells with an abortion of about 50 percent of pollen grains was observed also in the scion when *Nicotiana Langsdorffii* was grafted on *Solanum nigrum*. Regular spindles were seldom observed in the heterotypical division of *Nicotiana Langsdorffii* scion. The chromosmes were irregularly spread in the pollen mother cells as in species hybrids. Irregularity in second division was observed also. In the plant from which originated

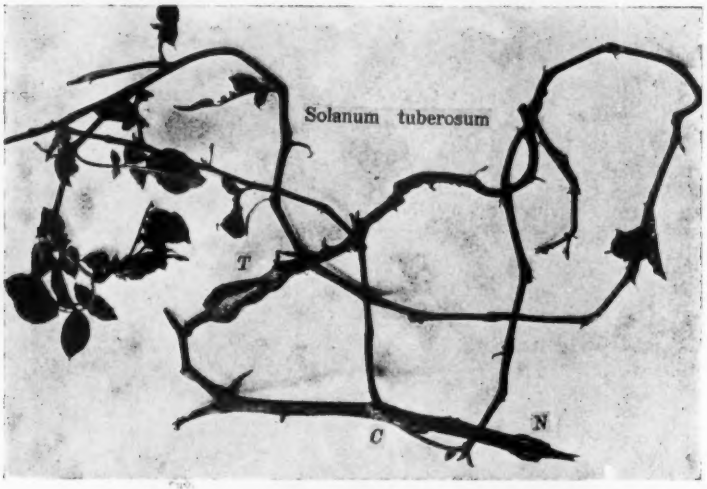

FIGURE 11.—Solanum tuberosum grafted on *Nicotiana Sanderae*. T—tuber, C—callus, N—*Nicotiana Sanderae* stock.

the scions, growing in the same environmental conditions, was observed normal cell division and no abortive pollen grains. The destruction of corolla and of calyx leaves and the disturbance in the pollen mother cell division are undoubtedly due to the stock activity.

In some graft unions the mutual reactivity of scion and stock is indicated by an agglutination of the plastids on both sides of the callus. In figure 9A are shown agglutinated chloroplasts of *Nicotiana glauca* grafted on *Capscium pyramidale*. Figure 9N shows normal chloroplasts of the same scion at a greater distance from the callus. Agglutination of chloroplasts was also observed in the unions *Solanum tuberosum* grafted on *Nicotiana*

Sanderae and *Solanum nigrum* grafted on *Nicotiana Sanderae*. The effect of agglutinin upon some of the cell elements is evident in a small area around the callus. The agglutinated zone spreads very slowly. In some cases the agglutination process results in death of the tissues.

The behavior of the starch in the graft unions is of particular interest. A very striking phenomenon was observed in the union *Solanum tuberosum* grafted on *Nicotiana rustica*. In this union, the scion of *Solanum tuberosum* formed aerial tubers (figure 10) near to the callus. The starch components produced in the scion were accumulated and dehydrated in the aerial tubers in form of starch grains. Although the branches of tobacco stock required further nutrition such as was accumulated in aerial tubers (the stock had branches with capsules in which very weak and small seeds were formed) they did not utilize the materials formed in the potato. When the top of the scion was cut just above the aerial tubers, the nutrients accumulated in the scion were not utilized by tobacco; instead, new shoots arose at the top of the tubers (figure 11). There are two possible explanations for the accumulation of starch above the callus. Either (1) the enzymes (the mobilizators of the starch) are inactivated by antibodies produced in the stock against the specific substances of the scion, so that the dehydration process is accelerated, or (2) the starch components have a specific antigenic stereochemical nature and are attacked directly by the antibodies coming from the stock, thus causing the dehydration. It is not impossible that both processes are involved.

REICHERT (1913), concerning the specificity of starch, says: "In the hemoglobin research it was shown that the hemoglobin molecule is modified in specific relationship to genus, species, etc., which may be taken to mean that the form of protoplasm that is expressed by the term erythrocyte is correspondingly stereochemically modified; with starch it has been found .... that the molecule is likewise changed in specific relationship to genera, species, etc., which accordingly may also be taken to mean that during synthesis the products of activity are altered in their molecular peculiarities in specific relationship to the sterochemic modifications of the forms of potoplasm which produce them."

Starch can bo hydrolyzed and its derivatives again synthesized into starch grains. In these processes is there a despecification and later a specification, or are the hydrolyzed components of each particular starch specific? Further investigations are needed to answer this question.

In the unions *Nicotiana glauca* grafted on *Capsicum pyramidale*, *Nicotiana Tabacum* grafted on *Datura Wrightii*, *Nicotiana Tabacum* grafted on

Solanum Melongena, *Solanum Lycopersicum* grafted on *Nicotiana glauca*, *Nicotiana Tabacum* grafted on *Solanum Lycopersicum*, and *Solanum nigrum* grafted on *Nicotiana Sanderae*, an abundant accumulation of starch grains was also observed above the callus. Figure 12 represents the callus union *Solanum Lycopersicum* (L) grafted on *Nicotiana glauca* (C). In some species graft union—as *Nicotiana glauca* grafted on *Nicotiana rustica*, *Nicotiana Tabacum* grafted on *Nicotiana rustica*, *Nicotiana Rusbyi* grafted

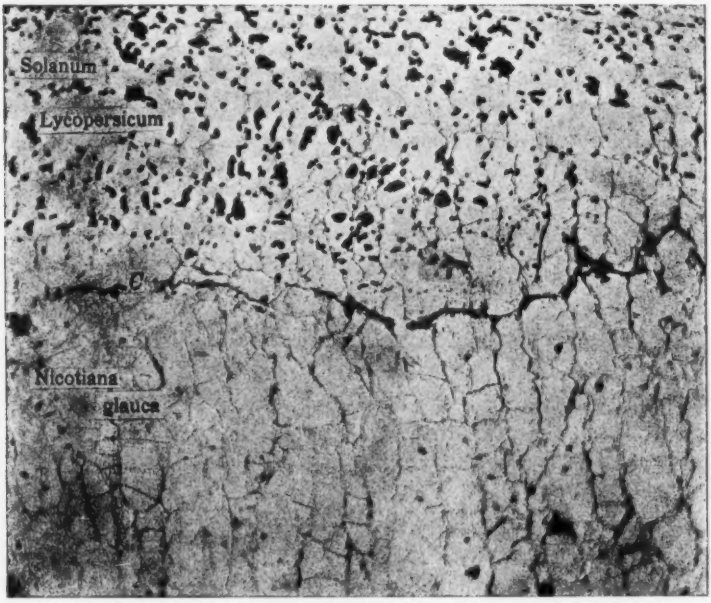

FIGURE 12.—Longitudinal section through the callus of the graft union—*Solanum Lycopersicum* grafted on *Nicotiana glauca*, stained with iodin. C-callus. *Solanum Lycopersicum* (scion) tissue contains abundance of starch, *Nicotiana glauca* tissue (stock) contains no starch near to the callus.

on *Nicotiana rustica*, etc., the stocks sometimes lost all leaves and the unions (that is, the scions) grew over 3–4 months without any great evidence of injury. These observations show that a part of the nutrients produced in the scions can be utilized by the stocks, apparently after a change when in passing the callus.

The physiological phenomena, expressed in morphological formations in the callus after an interaction between scion and stock, are in the same

way morphologically represented in the species embryos after an interaction between the mother plant and the embryo of hybrid nature. This parallelism leads to the conclusion that the antibodies produced in the mother plant are responsible for the characteristic phenomena observed in the species embryo development.

This conclusion is supported also by the precipitin reaction obtained between the extract of placenta of *Nicotiana rustica* plants on which have grown seeds of hybrid nature—*Nicotiana rustica*×*Nicotiana Langsdorffii*—and the normal extract of *Nicotiana Langsdorffii*. In 9 such tests the precipitin reaction "trace" was obtained four times, while in 9 other tests no precipitin reaction was observed between *Nicotiana Langsdorffii* normal extract and an extract of *Nicotiana rustica* placenta after selfing.

SUMMARY

1. Normal precipitins and lysins were found between many plant extracts of the Solanaceae.

2. In grafting certain species and genera of the Solanaceae, mutual induction of antibodies in scion and in stock was found.

3. The acquired immunity in such plants was tested by precipitin reactions.

4. The induced antibodies were specific in certain species.

5. Heterogenetic (non-specific) precipitins were also found.

6. The reactivity of the plants in acquiring immunity is, to a certain extent, an individual character.

7. The tested plants acquired the highest precipitin potency about between 30 and 45 days.

8. In some graft unions no precipitins were found..

9. In certain graft unions, which showed relatively high normal precipitins, a decrease of precipitins after grafting was observed.

10. No correlation between hydrogen ion concentration and precipitin reaction was found.

11. The effects of various physical and chemical agents on the antigenic and the immune extracts were observed.

12. High temperature inactivates both extracts.

13. Intensive light and optimum growth temperature increase the production of antibodies.

14. An agglutination of plastids near the callus was observed in certain graft unions.

15. In some intergeneric graft unions irregular cell divisions in the pollen mother cells were found. Abortive pollen grains were also observed.

16. Physiological interaction between scions and stocks accompanied by different morphological phenomena in the scions and stocks were observed, which are explicable by postulating antibody production.

17. Starch accumulated above the callus fails to pass down into the stock.

18. A parallelism was drawn between the interaction of scion and stock and that between mother plant and embryos of hybrid nature.

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NOTES ON THE SOMATIC CHROMOSOMES OF THE SEVEN-CHROMOSOME GROUP OF FRAGARIA

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INTRODUCTION

In continuing the work of MANGELSDORF and EAST (1927) and of ICHIJIMA (1926) on the collection of Fragaria at the BUSSEY INSTITUTION, it was thought desirable to make a more detailed study of the somatic chromosomes. The different Fragaria species have been found to fall into three groups according to their chromosome number by LONGLEY (1926), and by ICHIJIMA (1926). The basic chromosome number for the genus is seven. The species thus far reported in the diploid group are: *Fragaria vesca* (a number of varieties), *F. californica*, *F. mexicana* and *F. bracteata*. Five more species are now to be added to this group.

MATERIAL AND METHOD

Plants of the above mentioned species used are from the stock described by MANGELSDORF and EAST (1927). The following additional diploid species are under study: *Fragaria collina*, *F. maxima*, *F. nilgerrensis*, and two other species as yet unidentified. Another plant received from Albert Etter in California under the name of *F. Duchesnea* has fourteen somatic chromosomes. He states that it came originally from China. It corresponds very closely to our specimens of *F. nilgerrensis*.

Fragaria collina Ehrh. The description of EHRHART (1792) is brief: "Calyx fructus erectus. Folia acute serrata, utrinque pubescentia." "Serraturae ovato-lanceolatae; extima proximis brevior. Pubescentia petiolorum patentissima, pedunculorum erecta." The leaflets are longer in comparison to width and more acute than is found in the other seven-chromosome species. The petioles are moderately long. The leaves are

somewhat thinner than those of *F. virginiana*. The flowers are white, hermaphroditic and rather small; but larger than those of the *vesca* type. It is indigenous to central Europe. Our plants were obtained from Vilmorin, Andrieux and Company, Paris.

F. maxima is very similar to *F. collina*. The leaves are possibly somewhat larger, but the general appearance is much the same. The plants are believed to have come from the botanic garden at Tiflis, Russia.

F. nilgerrensis Schlecht. In leaf and flower shape this species shows a similarity to certain varieties of the 28-chromosome species, *F. chiloensis*. The leaflets are obovate, of moderate size, with a heavy pubescence especially below and on the leaf-stalk. The flowers are large; the petals white with a pink blush. The scapes are short, carrying three to four flowers. The stamens are much shorter than the large receptacle. These plants came to us from the UNITED STATES DEPARTMENT OF AGRICULTURE and were originally obtained from Vilmorin Andrieux and Company who say of them:

"This form is close to *Fragaria nilgerrensis*; it has the same foliage and the same small white fruits with darker seeds, but it is more vigorous, and the fruits might be considered as edible, whereas in the type their taste is unpleasant."

It is a native of India and Southeastern Asia, in moutainous and temperate regions.

Fragaria sp. (FPI 64856).Plants under this label were received from Mr. G. M. DARROW of the UNITED STATES DEPARTMENT OF AGRICULTURE last year. He states that they "were grown from seed which Mr. DORSETT purchased in a market in Hingan, Manchuria, the fruits having been gathered in the hills nearby."

The plants somewhat resemble the *collina* type, but the leaflets are much less acute and the scapes are much shorter. The leaves are darker green than those of either *F. vesca* or *F. collina*. The flowers are of moderate size and are white, tinged with yellow at the base of each petal.

Fragaria sp. (429) Two Hawaiian species were kindly sent to us by Doctor A. J. MANGELSDORF. The one given the stock number 429 proved to be diploid. The flowers are similar to those of *F. vesca*, being small and white. The scapes are about the same length as the leaf stalks. The fruit is white, almost acute in shape. The seeds are prominent and are straw-colored. The leaves are large, the margins coarse-toothed. They are scantily pubescent and moderately thin in texture. The leaf stalk is heavily pubescent.

Explanation of Plates

The first 22 figures show methaphase chromosomes; the last six show prophases. All are taken from somatic divisions in the root tips.

Plate 1

Figure 1.—*F. vesca* (from Ecuador).

Figure 2.—*F. mexicana.*

Figure 3.—*F. bracteata.*

Figure 4.—*F. collina.*

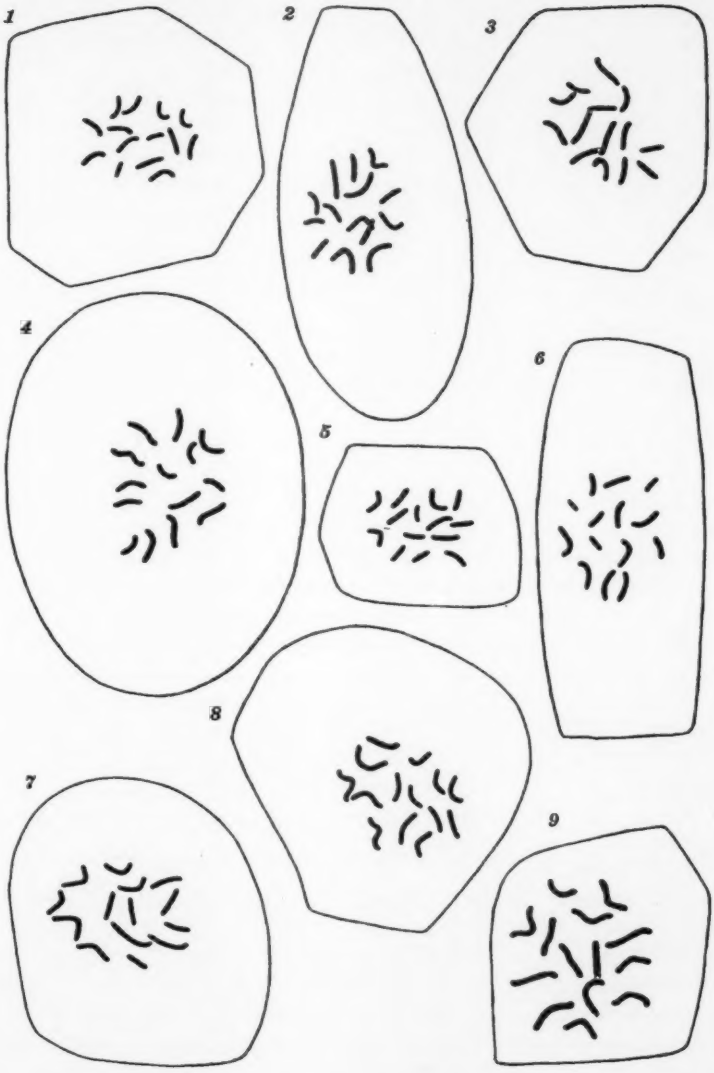
Figure 5.—*F. maxima.*

Figure 6.—*F. sp.* (FPI 64856).

Figure 7.—*F. Duchesnea.*

Figure 8.—*F. Californica.*

Figure 9.—*F. nilgerrensis.*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Root tips were fixed in Flemming's strong and weak solutions good results being obtained with both. Other fixatives such as Bouin's solution, its modification by Allen, Carnoy's solution, etc., proved unsatisfactory, although ICHIJIMA reports good results with Allen's formula. The sections were cut from 6 to 8 microns thick, usually the latter. Haidenhain's iron-haematoxylin was used in staining. All drawings were made with the aid of the camera lucida, using a 1.5 mm. Spencer apochromatic objective; tube length 160 mm. Figures 23 to 29 were drawn with a ×10 ocular, the rest with an ×18.

SIZE RELATIONSHIPS AMONG THE CHROMOSOMES

An attempt was made to find constant differences in chromosome shape. Similar shapes were found in many cells, but they apparently were not confined to chromosomes of the same relative length. Two chromosomes of about the same length in a cell often show a striking similarity in shape (See figure 7).

The only difference among the chromosome pairs that remains at all constant is that of relative length. Even size differences are not always clearly apparent. The small size of the chromosomes, their shape, and often their position, make an estimate of length difficult. On the whole the chromosomes of the different species are very similar considering their wide geographic distribution, the diversity of their morphological characters, and their breeding behavior.

The seven pairs can be arranged into an evenly graded series. Measurement with an ocular micrometer of fourteen metaphase plates gives the following average lengths in microns: 1.7; 1.5; 1.4; 1.3; 1.2; 1.0; 0.9. It is usually easy to distinguish the two shorter ones and at least the longest pair. The measurements with the ocular micrometer were checked from the drawings by the use of a scale made with a camera lucida from a stage micrometer at the same magnification as the drawings.

ASSOCIATION OF CHROMOSOME HOMOLOGS

Somatic association of homologs seems to be most complete in the Diptera (METZ 1916). It has, however, been noted in numerous plant genera (STRASBURGER, CL. MÜLLER, STOMPS, NEWTON, OVERTON and others). The most recent observation that has come to my attention is that on *Zea mays* by FISK (1927). She seems to have found much the same situation in her material that is evident in Fragaria. While pairing is unmistakable it is far from universal. What may be seen in Fragaria is a frequent association of chromosomes not only of equal or nearly equal length, but very

PLATE 2

FIGURES 10–19.—The chromosomes of the first nine figures are here arranged in pairs according to length.

FIGURE 20.—*F. nilgerrensis*, showing end-to-end arrangement of the chromosomes. Fifteen chromosomes can be counted.

FIGURE 22.—*F. sp.* (429).

FIGURES 23–25.—*F. nilgerrensis*. showing the tendency for association in the prophase. Figures 24 a and b are from the same cell.

FIGURES 26 and 27.—*F. collina*.

FIGURE 28.—*F. vesca*. None of the prophase figures (23–28) show the complete chromosome complement.

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often of similar shape. In some cells this can be seen for all of the chromosomes (figure 22), more often for four or five pairs. Occasionally no pairing can be detected. These observations are based on an examination of well over a hundred metaphase plates. The first nine figures were not selected to show pairing. The relationship may be either end-to-end or parallel.

Figure 20 gives the suggestion of a continuous end-to-end arrangement, but if this is the normal occurrence it is a stage of very short duration. There was also evidence of an end-to-end arrangement in *Zea mays*. "Many cells were found in which a number of the chromosomes were arranged end-to-end as though they had just been separated by the segmentation of a spirem." (FISK 1927, p. 58).

An examination of the prophases for "pairing" is complicated by the facts that the chromosomes are not in a convenient position for observation and that they do not differentiate as clearly as in the metaphase. I did not find the sort of association here as reported by STOMPS (1911) working on *Spinacia oleracea* and by NEWTON (1924) on Galtonia; but there is some evidence of attraction as shown in figures 23 to 29. This association seems to be due not to a mechanical attachment holding over from an early spireme stage but to a mutual affinity.

The writer wishes to acknowledge the kind help and guidance of Doctor E. M. EAST in carrying on the Fragaria studies. Thanks are also due Doctor A. J. MANGELSDORF and Professor M. CHRISTOFF for assistance during the early part of the work.

SUMMARY

1. Five new species, *Fragaria collina*, *F. maxima*, *F. nilgerrensis* *F. sp.* (FPI 64856) and *F. sp.* (429) are added to the diploid group with 14 somatic chromosomes.

2. The somatic metaphase chromosomes form a graded series according to length.

3. Association of homologs in the somatic tissue is evident in all of the species of this group.

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GENETIC EQUILIBRIUM AND SELECTION*

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PRELIMINARY CONSIDERATIONS

Discoveries by Mendel and later scientists indicate the prevalence of a mathematical regularity underlying much of genetic behavior in inheritance. Before an intelligent evaluation of the various practices in plant and animal improvement may be made, it is essential to know more of the fundamental principles upon which these practices are based. It is essential to know more of this mathematical regularity, the extent to which it is operative, and the ultimate results of its action. Of equal value is a knowledge of the extent to which these results are desirable for utilization by man. The present study represents an effort to ascertain more of the underlying principles of plant and animal improvement.

Selection

One may logically concede that, in so far as genetic constancy exists selection cannot modify the characteristics of a population. It must have variability to play upon.

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The only types of sexual populations that remain constant under a given environment would appear to be: (1) a single homozygous biotype either inbred or random bred; (2) a homozygous inbred population, consisting of different biotypes in which all members reproduce equally;(3) a population held in equilibrium by (a) recurring mutation, (b) balanced detrimentals, or (c) a combination of these two. In the case of any other population selection occurs. The inevitable result of selection is approach toward uniformity as produced by one or more of the foregoing conditions. This approach toward uniformity has been discussed by HAGEDOORN (1921). More detailed mathematical consideration of some of the conditions underlying the phenomenon have been presented by R. A. FISHER (1922) and by WRIGHT (1921). The intensity of selection in any population depends upon: (1) the proportion of parents and (2) the variability in progeny number from individual parents. In a population of constant size the proportion of parents is influenced (1) by size of progenies, and (2) by degree of inbreeding. In an inbred form producing many descendants per parent, natural selection is intense; therefore a small proportion of the population is apt to engage in reproduction. In such a type uniformity should be approached with relative rapidity.

On the other hand, the proportion of the total population that engage in reproduction is probably greater in man than in any of the domestic plant or animal forms, hence the effects of selection should be most gradual in man.

As a result of the "Hagedoorn effect", which tends to eliminate factors in low frequency, an approach toward homozygosis may take place in a population, even when all factors are equally beneficial. This effect should be rapid when great numbers of progeny are produced by each parent or when a small proportion of the population engage in reproduction. This process should be more rapid when factors of a single allelomorphic series differ in their value. In such a case members of the population are variable in genetic vigor. Detrimental factors are eliminated from the population at a rate depending upon the facility with which they may be affected by the selective processes. Beneficial allelomorphs come into the ascendency while those that are detrimental follow a curve of elimination that may be asymptotic to zero. As frequency approaches zero, the curve of elimination becomes so nearly horizontal that it may be concluded selective processes exert little influence. However, under such conditions the "Hagedoorn effect" would appear either to throw out these factors in low proportion or to throw them higher up the scale of frequency where

selection is more effective. When a small proportion of the population engage in reproduction, a large proportion of factors in low frequency should be eliminated. Under such conditions those factors that are retained should be thrown into higher frequency where selective elimination is more rapid. It seems evident, therefore, that, other things being equal, the proportion of factors in low frequency is lower, and the mean level of frequency for each factor is higher when few individuals become parents.

Mutation

Factors that are produced by recurring mutation are not completely eliminated by the foregoing processes. It is possible that a beneficial factor may mutate to a detrimental or a detrimental factor may mutate to a beneficial one. However, since selective elimination should keep detrimentals in very low proportion in the population, there may be relatively few at hand to mutate; hence typical mutations would probably be detrimental. When inbreeding a normally cross-bred form, certain detrimentals approach a frequency of unity, hence it is possible that beneficial mutations in any allelomorphic series are more easily observed in such artificially inbred forms.

Reproduction versus production

In order that there may be no confusion of terms, it seems advisable to distinguish between reproduction and production. By reproduction is meant the relative number of progeny in which the germ plasm, through ovule or pollen, is distributed. By production is meant the yield in quantity or quality of that commodity of the plant that makes it economically valuable; as, grain of cereals, beauty of shrubbery, or luxuriance, palatability, and nutritive qualities of forage. It may be accepted as a general principle that the selective processes are either natural or artificial. Each one tends to reduce genetic variability, but in these two cases the end result may be different. Natural selection produces a highly reproductive plant; that is, natural selection tends to favor the type best able to propagate itself. On the other hand, intelligent artificial selection produces a highly productive one; that is, intelligent artificial selection propagates the type best able to yield economic goods for man. These economic goods may represent either quantitative or qualitative characteristics, and need not be associated with reproductive efficiency. Selection, then, represents the resultant of two forces, each limited in its scope by the heritable variability at hand and either one able, by itself, fully to utilize that variability. Natural selection is always in operation with reproductive effi-

ciency as its ultimate goal. In so far as the objective of artificial selection happens to favor reproduction, the two processes select for the same end product; therefore in such a case efforts at artificial selection yield little beyond that accomplished by nature. On the other hand, man's economic goods may be a product that is not associated with reproduction or that runs counter to it. Under such conditions, man's selection should be highly effective, but the natural processes may be opposed to his effort. When this occurs man must exercise incessant vigilance to maintain the population in its consequent unstable equilibrium.

It seems evident, then, that the processes of plant and animal improvement must depend upon: (1) the extent to which equilibrium exists in the population; (2) the reproductive behavior at equilibrium; and (3) the margin between reproduction and production.

Scope of present paper

The primary aim in this paper is to discuss the germinal purpose of selection. This purpose aims only to isolate germ plasm capable of the greatest production when properly selected. This paper is not interested in the somatic purpose of selection which aims to isolate mature, viable seed that will not convey diseases to the progeny. It is assumed as self-evident that somatic selection is frequently and necessarily essential. To plant decayed potatoes or frozen corn kernels would be absurd. To plant seeds infected with certain diseases might produce even more disastrous results. As considered in this study, germinal selection might be defined as that type of selection which induces the tendency toward a heavier production from seed on one generation than was possible from seed subjected to equally careful selection in a previous generation.

This paper is limited to a discussion of (1) some conditions that may induce equilibrium, (2) the reproductive behavior at equilibrium, and (3) the margin between reproduction and production. It makes no attempt to discuss the extent to which equilibrium exists in a population, the relative value of different agencies in inducing equilibrium, or the reproductive behavior of interacting factors when the result is different in kind from that of the constituent individual factors.

If we are to believe Darwin and his followers, the sum total of accomplishment by natural selection toward reproductive ends has produced important results. To show tangible effect where man's efforts parallel those of nature, his accomplishment must exceed hers. Knowing the merest fraction of her secrets, he must beat her at her own game. This is a strong

challenge. It is true that he may induce combinations which she has found it difficult to effect, but this process is outside the scope of selection and is productive only of material for selection to play upon. It does not follow that artificial selection is useless. It performs an important function aside from isolating specific germ plasm. However, there is probably no plant in which man's aim coincides with the result obtained by nature. But, an essential service for the breeder is performed if the field of usefulness of selection may be clearly defined, and if, for each group of plants, the different objectives are brought into relief.

The first division, then, that is made for purposes of the present discussion, separates plants into two groups; those grown for the production of vegetative products, and those for the production of grain. In the former, the overlapping of artificial and natural selection is at a minimum. In the latter, it is at a maximum. From this observation it follows that, in the former, the field of possible accomplishment by man is broad, while in the latter it may be more restricted.

Confining further discussion to those plants grown for the production of grain, it is clear that this group may be subdivided. In one division are those plants in which self-fertilization is the rule; in the other, cross-fertilization usually occurs. In the fromer, variability is too slight for nature to make as rapid adaptive progress as she can accomplish in cross-fertilized plants; hence, artificial crossing to induce variability has long been utilized. In cross-fertilized plants new seed is frequently introduced to increase the germinal diversity as material for selection. In an attempt to outline the margin between productive and reproductive ability within these groups, it is first desirable to observe the selective processes for reproduction in nature, and then to consider the selective processes for production by man.

THE SELECTIVE PROCESS FOR REPRODUCTION (NATURAL SELECTION)

It is now generally believed that the factors of heredity behave according to the laws formulated by MENDEL. Most cases with which one has to deal are too complex for any simple analysis, but, if the principle of this underlying regularity is accepted, many orderly results must follow. According to PEARSON (1904) a population composed of 25 per cent *AA*, 50 per cent *Aa*, and 25 per cent *aa* maintains these proportions in later generations under random-fertilization if all are equally reproductive. WRIGHT (1921) summarizes the work of HARDY (1908) in the following words: "There is equilibrium under random mating when the number of

heterozygotes is twice the square root of the product of the two homozygous classes." With a single factor pair, this condition is reached in the first generation after random mating begins. According to WENTWORTH and REMICK (1916), when many factors are involved, equilibrium is approached more slowly. Formulas for a number of genetic series are presented by JENNINGS (1916). Formulas presented by ROBBINS (1918) show the rate at which equilibrium is approached under different conditions of linkage. The rate at which complete homozygosis is approached under conditions of inbreeding is demonstrated by JENNINGS (1912).

In plant populations, however, it is usually not the individuals that are considered, but the proportion their product includes within the population or mass. Under such conditions, if the allelomorphs of a factor series possess unequal reproductive capacity, most of the foregoing formulas apply no longer. However, JENNINGS (1916) and WENTWORTH and REMICK (1916) present series showing the rate at which recessives and heterozygotes are lost from population when only dominants are selected for reproduction. WRIGHT (1921) showed the effect of selection toward a definite type.

The most extensive mathematical interpretation of selection that has appeared was presented by HALDANE (1924–1925). FISHER (1922) showed that when selection favors the heterozygote, a random-bred population comes into an equilibrium determined by the relative vigor of the classes present. His formula for equilibrium is expresses by WRIGHT[1] in the form $Q = \frac{L'}{L'+L''}$ in which Q = proportion of recessive gametes, $1-L'$ = vigor of homozygous dominant, 1 = vigor of heterozygote, and $1-L''$ = vigor of homozygous recessive.

Of particular interest in this connection is the following statement by FISHER: "If selection favors the homozygote, no stable equilibrium will be possible, and selection will then tend to eliminate whichever gene is below its equilibrium proportion; such factors will therefore not commonly be found in nature. If, on the other hand, the selection favors the heterozygote, there is a condition of stable equilibrium, and the factor will continue in the stock. Such factors should therefore be commonly found, and may explain instances of heterozygote vigor, and to some extent the deleterious effects sometimes brought about by inbreeding."

If the sum total of accomplishment by natural selection toward reproductive ends has been productive of important results, they have evidently

[1] (Not yet published; presented by permission).

been brought about by trying out different genetic combinations and discarding, with a lavish hand, from among the factors that are from time to time exposed to the selective agencies. And, if all natural forces within the plant are directed toward its numerical dissemination, those factors that are retained should be the ones from each series which contribute most to a highly reproductive plant. The only detrimental factors that escape the processes of elimination should be those so closely linked with beneficial ones that this sorting out can not take place. However, detrimental factors are apparently retained within a population. It is worth while, therefore, to consider the processes of factor elimination, the rate at which these processes may take place, and the conditions under which a factor may remain in equilibrium in the germinal complex and hence be a regular contributor to the genetic behavior of the plant population. Only from the results of such a consideration is it possible to judge accurately what type of factor complex may exist in the germ plasm, when artificial selection begins.

Some plants are wholly self-fertilized. Others are completely cross-fertilized. Many present degrees of intermediacy between these two extremes. Frequently the proportion of self-fertilization within a given variety or species varies, depending upon conditions external to the plant. Clearly it is beyond the scope of one short paper to present in detail the behavior for every different degree of intermediacy. It is considered sufficient, therefore, to deal first with conditions under self-fertilization, and then to consider conditions under random-fertilization. Complete cross-fertilization probably occurs nowhere else, except in the presence of self-sterility, dichogamy, or dioeciousness. For mathematical simplicity, studies are confined to those forms in which generations do not overlap. Among these it is desirable to know the rate at which a factor on any detrimental reproductive level disappears from the population. It is equally worth while to know the conditions under which equilibrium may be maintained to prevent the complete elimination of such detrimental factors.

Case 1. Under self-fertilization

To obtain a logical starting place for calculation, it is assumed that the genetic factor under consideration has been introduced through crossing or by mutation. In either case the immediate progeny is a heterozygote Such an isolated heterozygote is termed the first filial generation, or F_1. The second filial generation is composed of $\frac{1}{4}\ AA$, $\frac{1}{2}\ Aa$, and $\frac{1}{4}aa$. Let U, V, and W represent the reproductive capacities of AA, Aa, and aa respectively and R, S, and T represent the corresponding proportions. Then UR

represents the product of the reproductive capacity of AA multiplied by the proportion of the total population that it represents. The following formulas permit the proportions R′, S′, and T′ in any generation, to be derived from the preceding generation:

$$R' = \frac{UR + \frac{VS}{4}}{UR + VS + WT} \tag{1}$$

$$S' = \frac{\frac{VS}{2}}{UR + VS + WT} \tag{2}$$

$$T' = \frac{WT + \frac{VS}{4}}{UR + VS + WT} \tag{3}$$

When equilibrium is reached the propotion of R, S, and T remains constant from one generation to the next. From this fact it follows that, at equilibrium, $UR + \frac{VS}{4} = \frac{R}{S}\left(\frac{VS}{2}\right)$. This equation may be expressed in the form

$$V = \frac{4UR}{2R - S} \tag{4}$$

Also

$$V = \frac{4WT}{2T - S} \tag{5}$$

An examination of these values for V shows that a state of equilibrium occurs in which segregating heterozygotes are retained, only when V is more than twice as great as either U or W.

Since every member of the population is homozygous dominant, heterozygous, or homozygous recessive, it follows that R+S+T=1. According to (4) $V = \frac{4UR}{2R - S}$, hence $S = 2R - \frac{4UR}{V}$. Similarly $S = 2T - \frac{4WT}{V}$. Therefore $2R - \frac{4UR}{V} = 2T - \frac{4WT}{V}$ and $T = \frac{R(V - 2U)}{V - 2W}$. Substituting for S and T their values in terms of R, the formula "$R+S+T=1$" becomes

$$3R - \frac{4UR}{V} + \frac{R(V - 2U)}{V - 2W} = 1.$$

$$R=\frac{V(V-2W)}{V(V-2U)+V(V-2W)+2(V-2U)(V-2W)} \tag{6}$$

In like manner it may be demonstrated that

$$S=\frac{2(V-2U)(V-2W)}{V(V-2U)+V(V-2W)+2(V-2U)(V-2W)} \tag{7}$$

and

$$T=\frac{V(V-2U)}{V(V-2U)+V(V-2W)+2(V-2U)(V-2W)} \tag{8}$$

Doctor SEWALL WRIGHT has made the following suggestion: "These formulae seem to take the most instructive form if U, V, and W are broken up into portions necessary for maintenance of the size of the population and a uniform general factor (K) for rate of increase and also express one of the rates for maintenance as deviations from one. Thus let $K(1-U^1)$, $K(1-V^1)$, $K(1-W^1)$, be the reproductive capacities for AA, Aa and aa respectively. In the case of Aa, $(1-V^1)$ necessarily has the value 2, that is heterozygotes must double (under self-fertilization) to maintain their absolute numbers. Formulae for R, S, and T then take the forms:

$$R=\frac{W^1}{U^1+W^1+2U^1W^1} \qquad U=K(1-U^1)$$

$$S=\frac{2U^1W^1}{U^1+W^1+2U^1W^1} \qquad V=K(1-V^1)=2K$$

$$T=\frac{U^1}{U^1+W^1+2U^1W^1} \qquad W=K(1-W^1)$$

$$R+S+T=1 \qquad RU+SV+TW=K."$$

It becomes apparent from these formulas that R, S, and T for an inbred population at equilibrium may be determined if U, V and W are known.

To find U, V, and W, when R, S, and T are known, is equal value. Since $V=\frac{4UR}{2R-S}=\frac{4WT}{2T-S}$, it follows that

$$U=\frac{V(2R-S)}{4R} \tag{9}$$

and

$$W=\frac{V(2T-S)}{4R} \tag{10}$$

Let $V=1$. Then U becomes $\frac{2R-S}{4R}$. Also W becomes $\frac{2T-S}{4T}$.

Similarly, let $U=1$. Then V becomes $\frac{4R}{2R-S}$ and W becomes $\frac{R(2T-S)}{T(2R-S)}$.

The only conditions under which equilibrium may be established by these processes is when V is greater than 2 U and is also greater than 2 W.

Therefore, before equilibrium can be established for a segregating detrimental factor in an inbred population, the heterozygote must be more than twice as reproductive as either homozygote. Also, any factor whose heterozygote does not meet these requirements can be held in equilibrium in the population only by recurring mutation. As a corollary to this it is obvious that, in the absence of successive mutations, and when such excess reproductivity of the heterozygote is lacking, the only allelomorphs of a series that can be maintained in a mixed inbred population are those that are equal in reproductive value under the given environment. Thus, starting with equal proportions of AA and aa when the reproductive vigor of AA $=1$, and $aa=9/10$, the formulas for the n generation are $\frac{10^n}{10^n+9^n}=AA$, and $\frac{9^n}{10^n+9^n}=aa$. By the tenth generation the elimination process has proceeded until the population is composed of approximately 3/4 AA and 1/4 aa.

Finally, if all classes in the successive generations of any inbred genetic series that approaches equilibrium are multiplied by their reproductive values, the totals for the different generations are such as to permit the following general statement: When under inbreeding hybrid vigor is sufficient to maintain finite equilibrium, the reproductive capacity of the population as a whole continues to decrease from F_1 to equilibrium.

Case 2. Under Random Fertilization

The principles laid down by PEARSON (1904) and by HARDY (1908), as previously referred to, do not apply when all groups are not equally reproductive. The statement may be made, however, that whether equilibrium is maintained or not, under random-mating the number of heterozygotes is twice the square root of the product of the two homozygous classes. This holds for all cases except those in which the types differ in their sex ratio or pollen-ovule ratio. HALDANE (1924) showed that, when two allelomorphs are present in a population and are affected differently by selection, the favorable one comes into the ascendency and the detrimental one disappears at a rate that depends upon the effect of each and

the proportion in which they occur. The elimination curve of the detrimental is an S-shaped curve that approaches the horizontal at zero and at unity, but is relatively steep when the two factors are present in equal proportion.

One of the conditions that maintains equilibrium is equal reproductivity for all classes. Another condition that accomplishes the same result is compensating reproductivity for the different classes. The effectiveness of natural selection is based upon differences in reproductivity in any case.

In deriving genetic series under random-fertilization, when reproductive differences do exist, much laborious preliminary calculation is saved by using a few equations that suggest themselves from the behavior of factors under random breeding.

If U, V, and W represent the reproductive capacities of AA, Aa, and aa respectively, and R, S, and T represent the corresponding proportions, as is assumed in the series for inbreeding, it may be demonstrated that one random-fertilized generation of R′, S′, and T′ in terms of the preceding generation would be as follows:

$$R' = \frac{\left(UR + \frac{VS}{2}\right)^2}{(UR+VS+WT)^2} \tag{11}$$

$$S' = \frac{2\left(UR + \frac{VS}{2}\right)\left(WT + \frac{VS}{2}\right)}{(UR+VS+WT)^2} \tag{12}$$

$$T' = \frac{\left(WT + \frac{VS}{2}\right)^2}{(UR+VS+WT)^2} \tag{13}$$

At equilibrium $UR + \frac{VS}{2} = \frac{R}{S}(2WT+VS)$

or

$$V = \frac{2R(US-2WT)}{S(2R-S)} \tag{14}$$

Thus, under random-breeding, segregating heterozygotes are retained in the population if V shows any superiority over U and W. Since R+S+T =1 and since the proportion of each corresponds to that found in an expanded binomial it follows that $R = (1-\sqrt{T})^2$

$$S = 2\sqrt{T}(1-\sqrt{T})$$

Also $$UR+\frac{VS}{2}=\frac{R}{S}(2WT+VS)$$

To solve for $\sqrt{T}$ at equilibrium, substitute for R and S their values expressed in terms of T, The resulting formula may be simplified to

$$(1-\sqrt{T})(V-U)=\sqrt{T}(V-W)$$

$$\sqrt{T}=\frac{V-U}{2V-(U+W)} \tag{15}$$

Substitute Q for $\sqrt{T}$, $1-L'$ for U, 1 for V, and $1-L''$ for W. Then

$$Q=\frac{L'}{L'+L''}.$$

This is Fisher's formula as modified by Wright.

$$U=\frac{V(1-2\sqrt{T})+W\sqrt{T}}{1-\sqrt{T}} \tag{16}$$

$$V=\frac{U-\sqrt{T}(U+W)}{1-2\sqrt{T}} \tag{17}$$

$$W=\frac{\sqrt{T}(2V-U)-(V-U)}{\sqrt{T}} \tag{18}$$

(15) $$\sqrt{T}=\frac{V-U}{2V-(U+W)}=\frac{1}{2+\dfrac{U-W}{V-U}}$$

Let $U=1$, then $\sqrt{T}=\dfrac{1}{2+\dfrac{1-W}{V-1}}$

Let $\dfrac{1-W}{V-1}=H$, then $\sqrt{T}=\dfrac{1}{2+H}$ (19)

(21) $$W=\frac{\sqrt{T}(2V-U)-(V-U)}{\sqrt{T}}=1-\frac{\dfrac{1}{\sqrt{T}}}{(V-U)-\sqrt{T}(2V-U-1)}$$

When $U=1$, this formula becomes $W=1-\dfrac{\dfrac{1}{\sqrt{T}}}{(V-1)(1-2\sqrt{T})}$

Let $$\frac{\sqrt{T}}{(V-1)(1-2\sqrt{T})}=L.$$

Then $$W=1-\frac{1}{L}. \tag{20}$$

To find V in terms of H and L.

$$V=\frac{U-\sqrt{T}(U+W)}{1-2\sqrt{T}}$$

Let $U=1$

Then $$V=\frac{1-\sqrt{T}(1+W)}{1-2\sqrt{T}}=1+\frac{\frac{1}{1-2\sqrt{T}}}{\sqrt{T}(1-W)}$$

In the formula $H=\frac{1-W}{V-1}$, substitute for V its value $\frac{1-\sqrt{T}(1+W)}{1-2\sqrt{T}}$ and simplify to $H=\frac{1-2\sqrt{T}}{\sqrt{T}}$. Also substitute the same value for V in $L=\frac{\sqrt{T}}{(V-1)(1-2\sqrt{T})}$ and simplify to $L=\frac{1}{1-W}$

$$HL=\frac{1-2\sqrt{T}}{\sqrt{T}}\times\frac{1}{1-W}=\frac{1-2\sqrt{T}}{\sqrt{T}(1-W)}$$

But $$V=1+\frac{\frac{1}{1-2\sqrt{T}}}{\sqrt{T}(1-W)}$$

Therefore $$V=1+\frac{1}{HL} \tag{21}$$

By using the foregoing formulas it is readily demonstrated that, under random-fertilization, detrimental recessives are eliminated in the same manner as under self-fertilization, except at a slower rate. It is also apparent that all equilibrium series approach 1 as a limit just as they approach 2 under self-fertilization. Hence, to keep productivity unequal allelomorphs in equilibrium in a random-bred population it is essential that the heterozygote be more reproductive than either homozygote.

The processes involved in the approach to genetic equilibrium have been discussed. There remains for consideration the effect of these processes

upon reproduction of a population. If U = 1, V = 2, W = 0, a population comes into equilibrium at 4/9 *AA*, 4/9*Aa*, and 1/9*aa*. The reproductive capacity of this population is $(4/9\times1)+(4/9\times2)+(1/9\times0)=12/9=1.333$ The seed that produces such a generation is $\frac{1}{2}$ *AA* and $\frac{1}{2}$ *Aa*. Now change these proportions and use a seed mixture consisting of 3/8*AA* and 5/8*Aa*. The new progeny consists of 64/169 *AA*, 80/169 *Aa*, and 25/169 *aa*, while its reproductive capacity is 1.326. All possible similar changes produce a series of numbers. It starts at 1 for *AA*, rises to 1.333 for $\frac{1}{2}$ *AA* and $\frac{1}{2}$*Aa*, then drops to 1.25 for *Aa*. Thus for the population as a whole, the level of highest reproduction is obtained where equilibrium is established. Any other population similarly tested would bring about the same result. That genetic equilibrium is established in a random-bred population at the reproductive maximum is the inevitable conclusion.

Multiple Factors

The foregoing discussion demonstrates the behavior of individual factors in heredity when the factors of one allelomorphic series differ in reproductivity. That typical characteristics of plants and of animals are determined by many factors and not by one alone is becoming a generally accepted principle. In general, the behavior of single factors is usually applicable only to the extent that it contributes to multiple factor behavior. JENNINGS (1917) showed that the general tendency in multiple factors is the same as when only a single pair of factors is involved.

Linkage

The facts of heterosis and the detrimental effects of inbreeding indicate that beneficial vigor usually accompanies heterozygosis. If successive mutation is eliminated and if a difference in reproductivity is manifest in the factors of a series remaining in equilibrium, excess vigor in the heterozygote should be the rule. JONES (1917) explains this hybrid vigor on the basis of linkage between beneficial and detrimental factors. Evidently linkage is the most plausible explanation that has been presented for this phenonenon.

That "in random mating the effect of incomplete linkage between two factors is only temporary" was shown by ROBBINS (1918). He dealt with those cases in which reproduction is equal for all classes. For such cases any crossovers, no matter how few, cause the linked factors to reach the same equilibrium as if no linkage existed. Only the rate at which equilibrium is approached is altered by linkage. Hence complete linkage is the only case in which any other equilibrium is approached.

But, if such complete linkage is the rule, any experimental effort to measure dominance of factors in equilibrium should show excessive effect for factors influencing reproduction.

Conversely, experimental determination, that a reproductively important factor in the heterozygous condition is not favored by selection, demonstrates that the detrimental allelomorph either is in process of elimination from the population or is reproduced by recurring mutation. HALDANE (1927) demonstrates that under mutation the population is in equilibrium when

$$PU^3+(2P-Q)U^2+(P-2Q-K+KQ)U-Q+KQ=0.$$

In the foregoing formula P = proportion A to a mutations, Q = proportion a to A mutations, K = coefficient of selection, U = gametic ratio.

To illustrate the effect of complete compensation linkage, assume linkage between series A and B in each of which dominance is complete, but aa has a reproductive capacity of O, and bb has a reproductive capacity of $\frac{1}{2}$.

Thus $U\frac{Ab}{Ab}=1\times\frac{1}{2}=\frac{1}{2}$, $V\frac{Ab}{aB}=1\times1=1$, $W\frac{aB}{aB}=0\times1=0$. It is apparent that $V\frac{Ab}{aB}=2U\frac{Ab}{Ab}$. The following calculation shows where this population comes into equilibrium.

$$U=1$$

$$(20)\qquad W=1-\frac{1}{L}=0.\quad L=1$$

$$(21)\qquad V=1+\frac{1}{HL}=2.\quad H=1$$

$$(19)\qquad \sqrt{T}=\frac{1}{2+H}=\frac{1}{3}.\quad T=\frac{1}{9}$$

$$S=2\sqrt{T}(1-\sqrt{T})=\frac{4}{9}$$

$$R=(1-\sqrt{T})^2=\frac{4}{9}$$

Proof: $$(11)\qquad R'=\frac{\left(UR+\frac{VS}{2}\right)^2}{(UR+VS+WT)^2}$$

$$= \frac{\left[\left(\frac{1}{2}\times\frac{4}{9}\right)+\frac{1\times\frac{4}{9}}{2}\right]^2}{\left[\left(\frac{1}{2}\times\frac{4}{9}\right)+\left(1\times\frac{4}{9}\right)+\left(0\times\frac{1}{9}\right)\right]^2} = \frac{4}{9}.$$

The population in equilibrium consists of

$$\frac{4}{9}\ \frac{Ab}{Ab},\ \frac{4}{9}\ \frac{Ab}{aB},\ \frac{1}{9}\ \frac{aB}{aB}.$$

Let *AA* represent the presence of a rest period in seed of an annual such that it prevents germination before spring, and let *aa* represent the lack of a rest period. Such recessive seed may germinate in the fall and be killed. Let *BB* represent dense chlorophyll and let *bb* represent sparse chlorophyll. In the wild state the population produces seed from which the plants are 4/9 sparse chlorophyll bearers, 4/9 dense chlorophyll bearers, and 1/9 are killed by winter. The dense chlorophyll group is twice as reproductive as the sparse chlorophyll group, but the ratio between the two groups remains constant.

If such a plant population be brought under domestication with ripe seed gathered annually and saved for spring planting, the 1/9 *aB/aB* becomes reproductive and, because of dominant B, W *aB/aB* becomes 1. As a result R and S approach zero as a limit and T approaches unity, hence no equilibrium is reached until the entire population is *aB/aB*. The chlorophyll deficiency disappears and carries with it the rest period in the seed. This process takes place so rapidly that, by the expiration of one hundred generations after domestication, only about two plants out of one thousand can survive in the wild state.

More Complex Cases

Up to this point none of those cases have been considered in which interacting factors produce a result, due to their interaction, different in degree from that of the constituent individual factors. Since such cases are of frequent observation, their results need consideration. DEMEREC (1926) has found thirteen different factors for albinism in maize. In some crosses only one pair of factors determines albinism; in others two or three pairs are involved. Similar lethals have been found in many plants. In some two-factor cases a homozygous recessive of one set does not show detrimental effect except when it occurs simultaneously with a homozygous

recessive of another set. The formulas for such cases may be somewhat involved, but they are of simple derivation.

In a two-factor case of the type *AA BB*, U_1 represents the reproductive capacity of *BB* as U has represented the reproductive capacity of *AA*. Also R_1 represents the proportion of *BB* in the population as R has represented the proportion of *AA*. In such a case the type *AA BB* shows a reproductive capacity of UU_1 and a proportion in the population of RR_1. It may easily be demonstrated that, in terms of the Nth generation, the generation indicated by N+1 contains a proportion of *AA BB* that, under inbreeding, is found by expanding the formula (i) to

$$R'R_1' = \frac{\left(UR+\frac{VS}{4}\right)\left(U_1R_1+\frac{V_1S_1}{4}\right)}{(UR+VS+WT)(U_1R_1+V_1S_1+W_1T_1)}$$

Under random-breeding an expansion of formula (11) gives

$$R'R_1' = \frac{\left(UR+\frac{VS}{2}\right)^2\left(U_1R_1+\frac{V_1S_1}{2}\right)^2}{(UR+VS+WT)^2\,(U_1R_1+V_1S_1+W_1T_1)^2}$$

Further expansion of these formulas should apply to any compound case so long as the interacting factors produce the same result in degree as produced by the constituent individual factors. On the other hand, where there is a reproductive effect of the interacting factors different in degree from that produced by the constituent individual factors, it becomes necessary to add symbols for the different effect caused by this factor

TABLE 1

Reproductive capacity and proportion in terms of T for all classes of a dihybrid, random-bred, population.

TYPE	REPRODUCTIVE CAPACITY	PROPORTION	PROPORTION IN TERMS OF THE DOUBLE RECESSIVE
AABB	$\bar{U}$ (URU_1R_1)	$\bar{R}$	$[(1-\sqrt{T})\,(1-\sqrt{T_1})]^2$
AABb	$\bar{M}$ (URV_1S_1)	$\bar{D}$	$(1-\sqrt{T})^2\,2\,\sqrt{T_1}\,(1-\sqrt{T_1})$
AAbb	$\bar{N}$ (URW_1T_1)	$\bar{E}$	$(1-\sqrt{T})^2\,T_1$
AaBB	$\bar{P}$ (VSU_1R_1)	$\bar{F}$	$2\sqrt{T}\,(1-\sqrt{T})\,(1-\sqrt{T_1})^2$
AaBb	$\bar{V}$ (VSV_1S_1)	$\bar{S}$	$4\,(\sqrt{T}\sqrt{T_1})\,(1-\sqrt{T})\,(1-\sqrt{T_1})$
Aabb	$\bar{Q}$ (VSW_1T_1)	$\bar{G}$	$2\sqrt{T}\,(1-\sqrt{T})\,T_1$
aaBB	$\bar{V}$ (WTU_1R_1)	$\bar{J}$	$T\,(1-\sqrt{T_1})^2$
aaBb	$\bar{Z}$ (WTV_1S_1)	$\bar{K}$	$2\,T\sqrt{T_1}\,(1-\sqrt{T_1})$
aabb	$\bar{W}$ (WTW_1T_1)	$\bar{T}$	$T\,T_1$

$\bar{R}+\bar{D}+\bar{E}+\bar{F}+\bar{S}+\bar{G}+\bar{J}+\bar{K}+\bar{T}=1$

interaction. Let $\overline{U}$ represent any reproductive effect of the interaction of *AA* and *BB* that is different from that expected on account of the effect of U and U_1 acting individually. Also $\overline{R}$ represents the proportion of *AA BB* that was formerly represented by RR_1. Table 1 shows all nine types in the population with their appropriate symbols. This table also presents the proportion of each type in terms of the double recessive.

One table should be sufficient to illustrate the effect of interacting factors. Assuming a random-bred strain of corn that possesses two factors for albino seedlings, both of these factors must be homozygous recessive for albinism to appear. The factors are *a* and *b*. Only aabb is albino. In such a case $U=1$, $V=1$, $W=1$, $U_1=1$, $V_1=1$, and $W_1=1$. Bar letters are taken at one, except $\overline{W}$ which is zero. $T=.09$.

Then $$R=(1-\sqrt{T})^2=.49$$

$$S=2\sqrt{T}(1-\sqrt{T})=.42$$

$$T_1=.25$$

Then $$R_1=.25, \text{ and } S_1=.5$$

Also $\overline{T}=.09\text{X}.25=.0225$ of the population that appear as albinos. The entire population is constituted as follows:

$$\overline{R}=RR_1=.49\times.25=\ .1225$$
$$\overline{D}=RS_1=.49\times.5\ =\ .2450$$
$$\overline{E}=RT_1=.49\times.25=\ .1225$$
$$\overline{F}=SR_1=.42\times.25=\ .1050$$
$$\overline{S}=SS_1=.42\times.5\ =\ .2100$$
$$\overline{G}=ST_1=.42\times.25=\ .1050$$
$$\overline{J}=TR_1=.09\times.25=\ .0225$$
$$\overline{K}=TS_1=.09\times.5\ =\ .0450$$
$$\overline{T}=TT_1=.09\times.25=\ .0225$$
$$1.0000$$

Of this population .0225 do not reproduce; hence the reproductive population is .9775. The lost genes are .25 of *a* and .09 of *b*. The proportions of all types remaining in the reproductive population may be computed as follows:

$$T=\frac{.09-.0225}{.9775}=.069$$

$$S=\frac{.42}{.9775}=.430$$

$$R=\frac{.49}{.9775}=.501$$

$$T_1=\frac{.25-.0225}{.9775}=.233$$

$$S_1=\frac{.5}{.9775}=.512$$

$$R_1=\frac{.25}{.9775}=.256$$

In the next generation the proportion of albinos is–

$$\frac{\left(WT+\frac{VS}{2}\right)^2\left(W_1T_1+\frac{V_1S_1}{2}\right)^2}{(UR+VS+WT)^2\ (U_1R_1+V_1S_1+W_1T_1)^2}$$

Since the reproductive vigor of all types is unity, this formula becomes–

$$\left(T+\frac{S_1}{2}\right)^2\left(T_1+\frac{S_1}{2}\right)^2=\left(.069+\frac{.43}{2}\right)^2\left(.233+\frac{.512}{2}\right)^2=.0193$$

In one generation the proportion of albinos drops from .0225 to .0193. The T curve of elimination is asymptotic to zero while T_1 curve of elimination is asymptotic to a value between T_1 and T_1–T.

The foregoing illustration demonstrates the fact that, when the interaction of non-detrimental factors produces a detrimental result, the proportion of all contributing factors is reduced until one essential for the detrimental result is eliminated.

It seems clear then that not the fact of elimination but the rate is changed when a detrimental result is produced by the interaction of factors.

General Considerations

It has been recognized that a detrimental dominant can not maintain itself in a population because it can not be concealed or protected against selection. However, it apparently has not been so generally recognized that a detrimental recessive can not maintain itself under ordinary conditions. If the laws of heredity are mendelian, and if one allelomorph in a factor series be more reproductive than another, this one should come into the ascendency and the other should be wiped out. The rate depends

upon the facility with which the factor may be affected by the selective processes.

Unless equilibrium can be maintained in some way, only one factor of any allelomorphic series can persist in a population subjected to the action of natural selection. This principle is universal in both self-fertilization and random-fertilization. The only difference between the two types is manifested in the rate at which elimination takes place. However, different allelomorphs of a series apparently persist. It seems evident, therefore, that equilibrium can be established short of extinction for the supernumerary factors. If frequent factor mutation be barred, the only two conditions under which multiple allelomorphs of a single factor series may be maintained within any population are equal reproductivity of the factors and compensating reproductivity by the heterozygote.

It does not of necessity follow that the most reproductive allelomorphs of each series are the only ones remaining within the entire species, even though they may be the only ones remaining within a variety of that species in one environment. For others to be eliminated from the species, all possible combinations must have existed under each environment. It is possible that the slowly changing environment of a community may cause factors, once beneficial, to become reproductively detrimental. A modification of cultural practice or the advent of a disease may represent such an environmental change. But it does follow that, in the absence of frequent mutations, the usual condition includes single homozygotes, equally reproductive allelomorphs, or an excess reproductivity of the heterozygote over either homozygote. It is not impossible that the excess reproductivity of the heterozygote might be caused by something inherent in the allelomorphic series itself. A more plausible explanation assumes practically inseperable linkage of reciprocally balanced factors. It may be assumed, then, that through aeons many genetic combinations have been tried and have failed, while those able to persist are: (1) the factors without allelomorphs, (2) equally reproductive allelomorphs, (3) ever mutating beneficial factors, and (4) allelomorphs of different sets reciprocally balanced by linkage, or the beneficial member of one set completely linked with the detrimental member of another to give to the heterozygote the benefit of the best in both sets. Heterosis and the detrimental effects of inbreeding stand sponsor for the general presence of such reciprocal balance. But this condition is more difficult of attainment under self-fertilization, for under such a condition it has been shown that any equilibrium series approaches two as a limit. Therefore, the

heterozygote must be more than twice as reproductive as the normal dominant if equilibrium is ever to be maintained. Under random fertilization, on the other hand, any equilibrium series approaches one as a limit. Hence, finite equilibrium is maintained if the heterozygote possesses any appreciable superiority in reproduction. In any case, when equilibrium is attained under random-fertilization, it holds the genetic types in such proportion that reproduction for the population is at its maximum.

It is demonstrated that if a plant possesses a lethal factor in equilibrium—no matter how small the proportion might be—and if such plant be domesticated, this factor, if robbed of its lethal effect, will begin immediately to replace its normal allelomorph. Since the curve for such a procedure approaches unity as a limit, equilibrium is never established again until the normal type is completely obliterated. Hence lack of ability to persist in nature is not proof of long domestication. This fact leads to important conclusions, because by the operation to the principles underlying it one may expect that, when a plant or animal comes into such an environment that factors once detrimental are no longer so, bizarre forms may gain the ascendency and wipe out characteristics once essential to the existence of the species. This phenomenon may show a genetic basis for the old theory of loss by disuse.

THE SELECTIVE PROCESSES FOR MAN'S PRODUCTION OF ECONOMIC GOODS (ARTIFICIAL SELECTION)

An effort is made, in the first part of this paper, to present a picture of the probable germinal complex that exists in a plant after it has been subjected to aeons of natural selection in the wild state and under domestication. The final picture, before conscious interference of man has disturbed it, shows a complex of factors into which new allelomorphs are injected by crossing or by mutation. These new factors are struggling with the old for ascendency, but are usually eliminated or are held in low proportion by continued mutation. Some rise and fall with environmental fluctuations of the different seasons. Linkages drag others into positions of equilibrium that are unwarranted by the reproductive value of the factors themselves. Of many series, a single factor is all that can remain and such a factor is forever hidden from view of the geneticist. Other series contain two or more equally reproductive factors, but, if inbreeding is the rule, each remains homozygous in its own biotype. Since hybrid vigor and loss from inbreeding occur, it may be inferred that there is present another condition in which balanced detrimentals are maintained in equilibrium by linkage that should be practically complete.

Such linkage would cause the increased vigor of the heterozygote to hold detrimental factors in the germinal complex. Under self-fertilization only those cases persist in which the heterozygote is more than twice as reproductive as the more reproductive homozygote. Therefore, balanced detrimentals should be present in greater numbers under cross-fertilization in which this great reproduction is not required. Also, the hybrid vigor that accompanies their presence should be a more general phenomenon in cross fertilized forms.

It is assumed that such a condition is left by natural selection. There is present that germinal complex most conducive to reproduction under the given environment. However, man is usually concerned with reproduction only to the extent that it induces production of his economic goods. He seizes upon this natural state and endeavors to manipulate its forces to his own advantage. His possibility of further accomplishment depends upon the margin between his ideal and the result obtained by nature. In dealing with vegetative parts the margin between these two is broad, but, when dealing with quantity of seed, the margin may be exceedingly narrow. In the first case the two lines of development tend to diverge, in the second they tend to coincide. The methods that man has employed are simple selection and more complex breeding. Simple selection is the first to be considered.

SIMPLE SELECTION FOR SEED PRODUCTION

Simple artificial selection has a two-fold purpose. One is somatic, the other is germinal. The interest here is with the latter purpose. If natural selection isolates that germinal complex most conducive to reproduction, artificial germinal selection should reduce reproduction to the extent that it limits the field within which natural selection may operate. Hence, if any type of artificial selection does not accomplish positive results in increasing production in proportion to reproduction, it is probably detrimental.

For sexual reproduction pollen is essential. As an economic crop it is usually not worth considering. Under self-fertilization variation in pollen quantity makes little difference, if there is sufficient to fertilize all ovules of the flower that produced it. Any excess is wasted. Under random-fertilization the proportion that is utilized from any plant depends chiefly upon the proportion that the plant under consideration contributes to the air or insect carrier. Then, to the extent that cross-fertilization occurs, pollen quantity is a reproductive character, though not a productive one. Copious pollen and few ovules may make a plant as reproductive as sparse

pollen and many ovules, but the productive difference may be enormous. It is conceivable that difference in proportion between pollen and ovules on any reproductive level may be the result of one of the following possibilities:

(1) A single pair of equally reproductive factors may be the cause.

(2) Equilibrium may be maintained by linkage between factor sets for pollen and for ovules, or,

(3) An environment may prevent complete development of pistillate inflorescence and thus reduce its proportion.

Any one of these conditions may result in a wide variation between productive and reproductive efficiency; hence the equilibrium that nature has maintained may be altered to the benefit of man. To illustrate the first possibility, one may assume a single pair of equally reproductive factors with a difference in production. Of these factors A is conducive to the production of sparse pollen and many ovules, while a induces much pollen and few ovules. Neither is dominant.

$$UAA = \begin{cases} 1 \text{ for pollen} \\ 2 \text{ for ovules} \end{cases} \qquad VAa = \begin{cases} 1.5 \text{ for pollen} \\ 1.5 \text{ for ovules} \end{cases} \qquad Waa = \begin{cases} 2 \text{ for pollen} \\ 1 \text{ for ovules} \end{cases}$$

	F_1	F_2	F_∞	
RAA		$\frac{1}{4}$	$\frac{1}{2+\frac{\sqrt{10}}{1.5}} =$	$\frac{1}{4.10818}$
SAa	$\frac{2}{2}$	$\frac{2}{4}$	$\frac{\frac{\sqrt{10}}{1.5}}{2+\frac{\sqrt{10}}{1.5}} =$	$\frac{2.10818}{4.10818}$
Taa		$\frac{1}{4}$	$\frac{1}{2+\frac{\sqrt{10}}{1.5}} =$	$\frac{1}{4.10818}$

Equilibrium is maintained at almost 1/4 AA, 1/2 Aa, and 1/4 aa, in spite of the fact that AA produces twice as much grain as aa. The average production of the entire population is 1.5. If only AA be selected for further breeding, a population may be obtained with an average production of 2. Thus selection for sparse pollen increases the average production by one-third. This hypothetical case illustrates the principle that, under

such a condition, selection for sparse pollen may be selection for yield increase. This purpose is effective in so far as it utilizes the margin between productive ability and reproductive ability. It must be pointed out, however, that in isolating homozygous AA, the field of natural selection is restricted, and for this reason the actual production may drop below 2, particularly if AA is linked with a system of balanced detrimentals.

It is also possible that heavier planting of plants homozygous for the less productive *aa* increases the level of comparative production per unit area beyond the level of relative production per plant. The net yield increase probably lies between one-third, as calculated, and the gain due to substituting grain for the weight of excess anthers and pollen.

For consideration of the second possibility, namely that equilibrium is maintained by linkage, a factor A for much pollen is linked with a factor b for few ovules, and vice versa. If $U\frac{Ab}{Ab}=\begin{cases}1 \text{ for ovules}\\ 2 \text{ for pollen,}\end{cases}$ $V\frac{Ab}{aB}=\begin{cases}1.5\\ 1.5\end{cases}$ for ovules for pollen and $W\frac{aB}{aB}=\begin{cases}2 \text{ for ovules}\\ 1 \text{ for pollen,}\end{cases}$ the first group is relatively twice as productive of pollen as of ovules; the second group produces equal proportions of each; while the third group is twice as productive of ovules as of pollen. Therefore, the situation is similar to the first condition. It is possible to assume any number of other situations in which the reproductive vigor of Ab/Ab may differ widely from that of aB/aB. For any of these cases equilibrium may be maintained at almost any level, if there is a degree of dominance in one or both linked factors to make Ab/aB sufficiently more reproductive than either homozygous class. Here again, artificial selection for many ovules or for sparse pollen raises the reproductive value of aB/aB and permits it to eliminate Ab/Ab in the same manner that the winter rest period is eliminated in the hypothetical case of the plant under domestication which becomes unfit for existence in the wild state.

Finally, it is necessary to consider the third possibility, an environment in which the total inflorescence of some plants can not develop to completion. Under such a condition staminate inflorescence may develop to completion while the pistillate inflorescence may be too abundant on some plants for the environment to permit of a full set of fruit or of seed. An altered ratio between pollen and functioning ovules is the result. This ratio is increased by abundant inflorescence or by unfavorable environ-

ment. When such a condition exists, the plant that produces a heavy yield, and is suppressed at the same time by reproductive competition, should be a plant with total inflorescence sufficiently sparse so that all ovules may develop.

A concrete illustration of the third possibility should make clear this action. For this illustration it is necessary to make an assumption that has not been verified. The assumed premise asserts that, since they are homologous organs, there are characteristics of the maize ear associated with like characteristics of the tassel.

If the assumed premise is valid, then the conclusion must be that a plant bearing a tassel with many rows of branches or of inflorescence and close-set flowers, should bear an ear with many rows of close-set kernels. Likewise, a plant bearing a tassel, sparsely branched with wide-spaced flowers, should bear an ear with few rows of wide-spaced kernels.

In a corn field of normal fertility all pollen is usually shed from every flower on the tassel, but all ovules on the ear develop into kernels only when the number is small enough so that the plant is able to develop each ovule. An ear with many rows of close-set kernels is likely to carry an undeveloped tip that may contain a large proportion of the total pistillate inflorescence. On the other hand, the ear on which the total inflorescence is small develops completely. Therefore, the pollen-ovule ratio changes when the environment prevents the development of all ovules.

If the foregoing assumption proves to be true, and if it is desirable to increase yield by utilizing the margin between production and reproduction as influenced by the effect of environment upon pollen-ovule ratio, the type of ear to select for poor soils would be the ear bearing few rows of thickened kernels. A more fertile soil is able to develop a more abundant total inflorescence, hence these ear characters should tend to lose their importance on such a soil.

It is not to be assumed that the foregoing statements represent unqualirecommendations of characteristics to select for in corn. They merely represent logical deductions, if the assumption in the premise be true. It is of interest, however, to note the close agreement of these conclusions with the actual findings from individual ear tests at different places in this country. Results of earlier tests are summarized by RICHEY (1922). Later results have since appeared (KYLE and STONEBERG (1925), GARRISON and RICHEY (1925), and RICHEY and WILLIER (1925). Unpublished work by ROTHGEB at the MARYLAND STATION indicates that tassel characteristics are associated with ear characteristics and with yield.

When plant selection is practised in cross-fertilized populations, for the purpose of increasing yields, the method used is based upon the assumption that unaided natural selection can not accomplish the desired result, whether it be permanent or only temporary. It seems clear, therefore, that the selective processes should modify the germ plasm in its content of equally reproductive allelomorphs that differ in production, or in its content of balanced detrimentals.

Such plant selection should be productive of permanent beneficial effect in only those cases for which the controlling factors are reproductively equal, but differ in productive capacity. Even in such cases, if artificial selection is not carried so far as to completely eliminate the unsirable factor, the result will be only temporary and, after selection ceases, the former equilibrium will be restored.

Among the balanced detrimentals, under cross-fertilization, selection for yield should also have only a temporary effect. Any benefit that results should be manifest on account of natural or artificial elimination of undesirable plants, or on account of the tendency for a plant in competition in part to make up for the deficiencies of its neighbors. All of these processes, whether artificial or natural, may be designated by the term "Selective Progeny Elimination."

As an illustration of the fact that reproductive benefit should not result from such selection, independent of progeny elimination $U\frac{Ab}{Ab}=\frac{1}{2}$, $V\frac{Ab}{aB}=1$, and $W\frac{aB}{aB}=0$. At equilibrium, this population consists of $\frac{4}{9}\frac{Ab}{Ab}$ $\frac{4}{9}\frac{Ab}{aB}$ and $\frac{1}{9}\frac{aB}{aB}$. Assuming that the factors designated by A and B are of such a nature that production equals reproduction, the production of the population is:

$4/9\times\frac{1}{2}=4/18$
$4/9\times 1=8/18$
$1/9\times 0=0$

Total $=12/18$ or .667

By selecting only seed of the most vigorous plants for reproduction, that is, seed of Ab/aB, the resulting population becomes:

$1/4 \times \frac{1}{2} = 1/8$
$2/4 \times 1 = 4/8$
$1/4 \times 0 = 0$

Total = 5/8 or .625.

If lethal aB/aB is a zygote lethal, which prevents development of the seed possessing it, or if selective progeny elimination occurs so that surviving plants make up in part for deficiencies of their neighbors, yield of the unselected seed may reach as a maximum:

$4/8 \times \frac{1}{2} = 4/16$
$4/8 \times 1 = 6/16$

Total = 12/16 or .75.

By planting only seed of Ab/aB, the population becomes:

$1/3 \times \frac{1}{2} = 1/6$
$2/3 \times 1 = 4/6$

Total = 5/6 or .883.

The degree to which these conditions operate determines the degree to which yield of seed from unselected plants rises from .667 to .75 and seed from selected plants from .625 to .838. No consideration is given to the amount of self-fertilization that may take place in such a population, or to the effect of overtopping of Ab/Ab by Ab/aB. The reproductive values assigned to different types are the values at equilibrium. Presumably one gains what the other loses. This calculation is presented to show that, for those cases where production and reproduction are equal, artificial plant selection of breeding stock, among cross-fertilized grain crops, may rest upon a precarious genetic foundation.

The foregoing illustrations make it clear that there may be essential differences between production and reproduction, even when grain yield is the quality desired. However, the objection may be raised that only single sets or single linked sets of factors are dealt with. Many sets of factors, or many linked sets, may be contributory to such differences as those enumerated. It seems clear, though, that, if such a situation exists in one factor set or linkage set, it should exist to the extent that such set contributes to the ultimate result. Hence, to the extent that equally reproductive factors and compensatingly linked factors are the only al-

lelomorphs remaining in equilibrium, this situation should exist for the factors that contribute to differences in production.

There are other methods of approaching the problem, however, that do not deal with individual factors, but only with the gross results of their interaction. Kernel size serves for illustration. Figure 1 shows the margin between production and reproduction in this characteristic. On this chart the straight line labeled "Seed Size" slopes from 1 toward zero. The curved line of crop production extends in a horizontal direction until a seed size of .8 is reached, when it begins to curve downward. The repro-

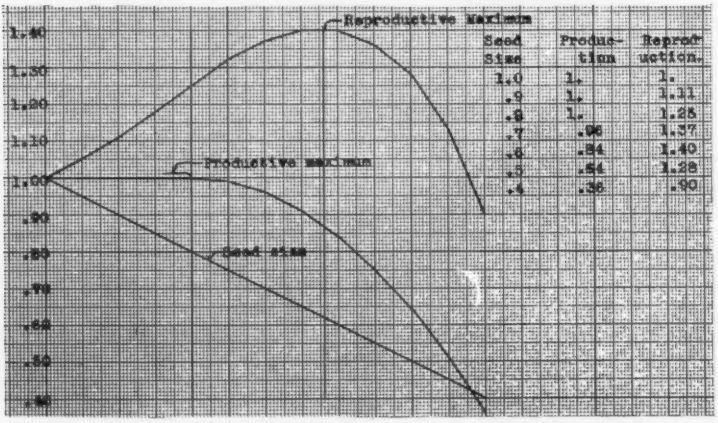

FIGURE 1.—In relation to production, reproduction is the reciprocal of seed size. The reproductive maximum, that is, the greatest number of progeny per plant, is produced beyond the point at which yield per plant begins to decrease.

duction line is the reciprocal of the "Seed Size" line; that is, a seed size of .9 divided into a production of 1 gives a reproduction of 1.11. Small kernel size is more reproductive than large kernel size so long as a reduction in kernel size does not cause a reduction in the number of seeds that develop from such a kernel. This is true because of more seed; hence greater numbers of progeny are produced from a given weight if the seeds are small. If the size continues to vary, this natural process should select smaller and smaller seeds until they become so small that the size, itself, becomes a productive factor. Even then the process may not cease, because the size for greatest reproduction may be smaller than the size for greatest production. It is evidently worth while to study more closely the processes involved.

At one extreme it is possible to imagine a seed so large that mere size is not a productive factor. Assuming such a size and such a production as unity, size may be reduced without reducing production, but reproproduction is increased. For, with the yield unaffected, and with the size dropping below unity, the numbers should rise above unity. When variability occurs, therefore, natural selection reduces size until production is affected by the process. It is highly probably—in fact practically certain—that, when production is affected by loss of size, the effect is at first slight and becomes more severe as size is further reduced; hence the line of production becomes a curve.

At the other extreme, because a seed is an organized resting plant, together with food material to nourish it through its early stages of growth, some size is necessary in the smallest seed that can function. If all gradations in size are considered, reproduction, or the growth of progeny from the seed, becomes zero before seed size becomes zero. Therefore, some characteristics of this curve of production are at hand. As seed size becomes smaller and smaller, production is in the early stages unaffected and reproduction is increased. Finally, a size is reached where production is progressively decreased, thus causing the line of production to curve downward more steeply as it approaches zero before the line of seed size reaches zero. The production line may approach the zero line abruptly, or it may flatten out forming an S shaped curve. This difference is immaterial for the present consideration, however, because only the early stages of the curve are of immediate interest. A curve of the shape $y = 1 - .01x^2$ possesses the essential requirements.

If the characteristics of the production curve have been correctly described, it must continue to rise until it reaches its maximum height at a point, in reduced seed size, appreciably beyond that where production begins to drop. Under natural selection, equilibrium is reached where the curve of reproduction is at its greatest height and seed size, in equilibrium, fluctuates about the corresponding value. Hence, selection for seed size greater than that at reproductive equilibrium should increase production. Figure 1 shows these curves. All begin at unity. Seed size is reduced at a uniform rate. The curve for production possesses the essential requirements that have been enumerated. At .8 for seed size, the production curve begins to drop. Since size is .8, and production is 1, reproduction is $1 \div .8 = 1.25$. This means that 1.25 units in number must be present per unit of volume if the size of each unit in number is .8. At the reproductive maximum size has dropped to about .62 and production has dropped to about .87.

For a simple illustration of the results of seed size, one may assume a self-fertilized plant in which one variety consists of equal numbers of two pure lines identical in all respects except seed size and the results arising from this difference in size. In figure 1 reproductive equilibrium is at a seed size of about .62. Productive maximum is not attainable below a seed size of .80. Assuming, therefore, that pure line A has a seed size of .62 and pure line B has a seed size of .80, the following table shows the proportions and yields of consecutive generations of such a variety:

TABLE 2

GENERATION	PROPORTIONS		REPRODUCTION	PRODUCTION
	A	*B*		
P_1	.50	.50	1.330	.935
F_1	.53	.47	1.335	.931
F_2	.56	.44	1.340	.927
F_∞	1.00	0.00	1.41	.87

It may be observed form table 2 that the larger number of progeny from the lower producing type is causing its proportions to increase, while production of the entire variety is decreasing. The population should ultimately consist of one type, A, and the production should reach. 87 instead of .935.

Therefore, in a mixed population, equilibrium is established at such a seed size that, within limits, artificial selection for increase in seed size should cause increase in production or yield. Here again the total expected effect may not be found, for disturbing factors need to be considered. For instance, when plants are grown under conditions of competition, large seed may give the plants from them a sufficient start to tend to overtop and hence injure their less vigorous neighbors. To the extent that such a condition is operative, reproductive equilibrium should move up the scale of seed size. Also, thicker planting of the smaller seed may make up for a part of the deficiency in yield per plant. On the average, however, it may be expected that artificial selection for large seed will result in increased yield per plant, even though in some cases the result may be overshadowed by other forces.

More complex breeding

The domesticated plants of a given species in a given geographical division may all have a common, recent origin, but the environment of that territory may present fluctuating or cumulative changes. Under such a condition it is possible that the local plants do not possess the germinal

complex best adapted to a typical environment to which the plant is subjected. Hence, the breeder searches for types new to the section, types probably not so well adapted as those already present, and endeavors by natural or by artificial crossing to bring about further variation in the local strains. If a disease has appeared to which all local strains are susceptible, the poorest and least adapted foreign type may possess the one necessary factor for resistance. By crossing and judicious selection, this one factor may be saved and the undesirable germinal material eliminated. After man has introduced the resistant factor, along with all of its detrimental companions, the question might be raised as to whether he can effect elimination better than nature can. The answer depends upon the completeness of his knowledge. For, given a segregating germinal complex, and excluding those rare cases where man knows the specific factors involved, together with their mode of inheritance, nature may make a better job of selecting for reproductive efficiency than can man. Apparently every genetic factor, reproductively detrimental under the conditions in which the plant is grown, will be gradually replaced if a more beneficial allelomorph is at hand. This process should operate under any type of fertilization, provided only that inseparable linkage with factors of reciprocal action is not present. Presumably any reproductively detrimental factor can be maintained in equilibrium in the population only by increased reproductive capacity of its heterozygote, or by frequent mutant origin, or by the interference of man. What man can accomplish he may bring about more rapidly than nature, and, in self-fertilized plants, he may isolate favorable biotypes that nature holds in subjection by the overtopping vegetation or early maturity of neighboring plants. However, the normal environmental fluctuation, which has aspects of time and place, and the ability for one plant to make up for the deficiencies of its neighbor, are so pronounced that the best general average yield obtained might be produced, not by a single uniform line, but by a mixture of these. If linkage is found under inbreeding such that hybrid vigor is produced greater than that of either homozygote, then it seems certain that man can maintain proportions in the progeny as of F^2 by persistent selection of the heterozygote and hold a higher reproductive level for the population than at equilibrium.

There remains one field of complex breeding that can not be overlooked. SHULL (1908) and EAST (1908) called attention to the detrimental effects of inbreeding normally cross-fertilized plants and the hybrid vigor of the first generation when inbred lines are crossed. Work based upon the sug-

gestions of these men has been carried further with maize than with any other plant. It has been definitely demonstrated that, when maize is inbred, many distinct uniform types appear and production is rapidly reduced. Some lines become so weak that they can not be perpetuated. However, on crossing two of the survivors that are reduced in vigor and almost uniform in type, the first filial generation exhibits a degree of vigor that approaches (equals, or sometimes exceeds) the vigor of the original varieties before inbreeding began. The second generation exhibits less uniformity and vigor. Practically all of the inbred strains show reduced vigor, which is apparently due to the interaction of many genetic factors. In some a single detrimental characteristic is apparent, while in others none are observed as entities. This loss of vigor in detrimental recessives has been attributed to homozygosis and hybrid vigor to the covering of all detrimental recessives by their normal dominants. In the second generation following the cross between inbred strains all detrimentals are not concealed by heterozygosis, because the plant might not be completely random-fertilized, and because under random-fertilization a large proportion of the factors that can segregate are homozygous in some plants. Hence, the first generation is the only one that manifests the full degree of hybrid vigor. Among the processes that have been recommended for utilizing this hybrid vigor in corn are (1) the remixing of inbred strains from among which the most detrimental recessives have been eliminated, and (2) the use of single or double crosses between approved inbred strains. This former process would aim to build up a synthetic variety which would lack many of the detrimentals that reduced yields in the parent variety. LINDSTROM (1920) suggested that, in eliminating these detrimentals, many beneficial factors might also be eliminated. In any case, these processes are so widely recommended as to justify careful study of their implications.

Evidence has here been presented to show that, barring mutation, segregating factors retained in equilibrium are equally reproductive allelomorphs and balanced detrimentals. These detrimentals may be slight, severe, or lethal in their effect. In maize, since the grain from small ears is not usually planted, any severe detrimental becomes lethal in effect. Therefore equilibrium is maintained as it would be if such severe detrimentals were lethal.

Any detrimentals that are exposed by inbreeding are those held in equilibrium, or the products of factor mutation that are not completely eliminated by natural selection. In the latter case these products in any

allelomorphic series must usually be present in small proportions. Their elimination only hastens a process already in operation, or produces a temporary result because of recurring mutation. Complete elimination of a detrimental held in equilibrium requires that the beneficial dominant over its balanced detrimental be also eliminated.

As illustrating the result of normal equilibrium, one may assume that aa represents an albino lethal in a maize population which normally shows five per cent of inbreeding in excess of that expected under random-fertilization, and that $R\frac{Ab}{Ab}=.95$ and $S\frac{Ab}{aB}=.05$ in the reproductive population. If all plants are inbred, five per cent of the ears will segregate for albino seedlings. The progeny of such a population shows slightly more than one albino plant in one thousand. To hold the population in this equilibrium it is necessary that the reproductive vigor of the heterozygote be greater than the vigor of the homozygous dominant. If $U\frac{Ab}{Ab}=1$, $V\frac{Ab}{aB}=X$ and $W\frac{aB}{aB}=0$, then $.95\left(UR+\frac{VS}{2}\right)+.05\left(UR+\frac{VS}{4}\right)=\frac{R}{S}\left(.95[2WT+VS]+.05\frac{VS}{2}\right)$ and $V=1.0533$. The total population is $.9487\frac{Ab}{Ab}$, $.0500\frac{Ab}{aB}$ and $.0013\frac{aB}{aB}$. To the extent that production equals reproduction, the yield rate is $.9487+(.05\times1.0533)$ or 1.00136, and if the surviving plants make up for the losses due to death of aB/aB the yield becomes 1.00267. The true yield probably falls between these figures.

On the other hand, if the lethal be eliminated by artificial inbreeding and selection, the synthetic variety contains only Ab/Ab of these linked allelomorphs and the yield of such a variety is 1.0. Thus, removal of the albino lethal causes removal of the linked beneficial that held it in the population and to that extent reduces the yield. In like manner, when the group exhibiting heterosis is the only one selected for reproduction, a yield loss occurs, unless prevented by other responses as overtopping or compensating growth. If only a detrimental be selected for reproduction, the result is too apparent to need demonstration. Then it appears that all types of cases necessary to confirm the former conclusion have been con-

sidered, and that natural selection brings the entire cross-fertilized population into an equilibrium with such a genetic balance that reproduction is at its maximum. If artificial selection increases or decreases the proportion of any genetic group, reproductive rate for the population is reduced. The differential rate that exists between the genetic classes should, if permitted, reestablish equilibrium on the former level of maximum reproduction.

There remains the latter process recommended for utilizing hybrid vigor in corn. It is the use of single or double crosses between inbred strains. Though the uniformity possessed by progenies of such crosses may be of much importance, only reproduction is considered here. Experimental evidence to date seems sufficient to justify the assumption that the hybrid vigor utilized in such crosses presents a very promising field for practical application. Only one phase of such work, however, touches the subject of this paper. It is selection of the inbred strains for crossing. Such crossing is designed to produce a completely heterozygous population. Apparently none of its value arises from the presence of single factors of any allelomorphic series, nor is its benefit to be expected on account of allelomorphs that are equal in productivity. The group of factor combinations that it seeks to utilize are the linked heterozygotes that hold the balanced detrimentals in equilibrium, or factors not directly concerned with reproduction. The degree of excess productivity possessed by these linked heterozygotes evidently measures to a considerable extent the maximum benefit to be derived from such a process. There are two conditions that are most important in determining the degree of such excess vigor within any linked set. One is the degree of detrimental effect of the factors to be hidden, the other is the proportion in which these detrimental factors are held in the population. Against these must be balanced the proportion in which heterozygous grouping is possessed by the normal population. If much hybrid vigor is already possessed, little can be gained by complete heterozygosis.

Hybrid vigor (V) increases directly with T and inversely with W. This is expressed by the formula $V = 1 + \frac{1}{HL}$, when $\frac{1}{HL} = \frac{1}{\frac{1}{\sqrt{T}} - 2}(1 - W)$.

The proportion (S) in which the heterozygous grouping is possessed by the normal population is found by the formula $S = 2\sqrt{T}(1 - \sqrt{T})$.

An illustration in which $T = 1/100$ and $W = 9/10$ produces a population

consisting of $.81\frac{Ab}{aB}$, $.18\frac{Ab}{aB}$, and $.01\frac{aB}{aB}$. The reproductive capacity of such a population is $.81+.18225+.009$ or 1.00125. An F_1 cross between $\frac{Ab}{Ab}$ and $\frac{aB}{aB}$ reproduces at the rate of 1.0125. Thus, the hybrid reproduction is $\frac{1.0125}{1.00125}$ or 1.0112 of that normally expected. The net heterosis or hybrid vigor is 1.12 per cent of random-bred vigor. Twice 1/L makes V become 1.025. The random bred population changes from 1.00125 to 1.0035 and the net heterosis is now .0214, or 2.14 per cent, instead of 1.12 per cent. Thus while heterosis or hybrid vigor changes directly with 1/L, the net gain of an F_1 cross between inbred strains can not be quite so rapid as the increase in severity of the detrimental character. It is clear, however, that, other things being equal, the greatest theoretical hybrid vigor will be obtained from crosses involving inbred strains in which the most detrimental allelomorphs are present. On the other hand, it is highly probable that the lethals and other seriously detrimental factors are present in very low equilibrium. Hence, factors with less detrimental effect may be present in greater proportion or higher equilibrium and consequently possess as great vigor in the heterozygote. In any case, the fact must not be lost sight of that any hybrid vigor possessed by the heterozygote of a lethal is lost to any F_1 hybrid between inbred strains because the homozygous strain can not be propagated.

Figure 2 is designed to present a graphical presentation of the interaction of T and W upon V. The diagonal lines follow the contours of hybrid vigor (V). It may be observed that vigor of the heterozygote (V) as shown at the bottom of the chart increases directly with T and inversely with W. One may assume, then, that the normal germinal complex under random breeding contains myriads of balanced detrimental factors that can not be observed as entities. Each of these factors is but slightly detrimental and may be in relatively high equilibrium. Along with these is an occasional severe detrimental or lethal in very low equilibrium. Because of the conditions that produce this difference in equilibrium, the typical lethal may manifest no greater hybrid vigor than is shown by the typical slight detrimental. However, since an occasional severe detrimental might be found in high equilibrium, it seems reasonable to expect that the most reproductive cross, if it is finally found, will be a cross between inbred strains so lacking in vigor that they may scarcely be reproduced.

Incidental selection

There are evidently many kinds of incidental selection. In fact it is difficult to distinguish between natural selection, on the one hand, and unconscious artificial selection on the other. To the extent that reciprocal balance exists in the genetic complex, a population comes into equilibrium in a given environment with the genetic factors of the different allelomorphic series present in such proportion that reproduction is at its maximum.

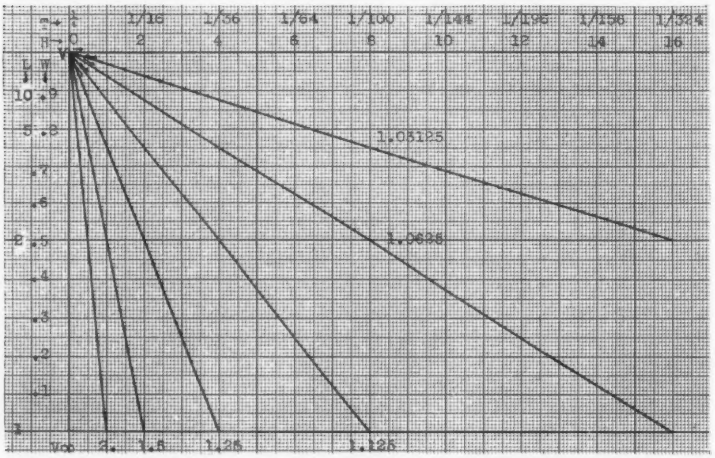

FIGURE 2.—Showing that in terms of unit vigor (U) of the homozygous dominant, heterosis or hybrid vigor (V) under random pollination increases with increase in the proportion (T) in which the detrimental recessive occurs in the population at equilibrium, and also with decrease in vigor (W) of the recessive. Note that high values for hybrid vigor (V) are relatively infrequent except when values (W) of the recessive are low.

It is clear, then, that a change in the environment tends to induce a new equilibrium on a new reproductive level. As a possibility of the changed environment, liming an acid soil might be cited. This treatment, with a plant population in its most reproductive condition, should induce such equilibrium because of the change in the soil. To the extent that liming creates an environment more beneficial to the plant, this new equilibrium should be on a higher reproductive level; therefore selection occurs as a result of liming.

As another example of a changed environment the chemical treatment of corn seed for "root rot" might be considered. Before determining the

beneficial results of such a process, it is necessary to find out whether resistance is hereditary. If it is not hereditary, seed treatment might be of value as a disease escaping process, but resistant strains could not be isolated. If it is hereditary, then there are three possible hypotheses dealing with this condition: (1) the disease is of recent occurrence, (2) susceptible plants are continually created by recurring mutation, or (3) factors for susceptibility are held in the population by reciprocal balance with other detrimentals. If the first hypothesis is correct, artificial selection can be of only temporary effect for the natural processes are tended towards elimination of susceptibility. If the second hypothesis obtains, artificial selection must be only temporary in its effect because recurring mutation re-creates the type selected against. If the third hypothesis is the correct one, artificial selection is detrimental unless it utilizes the margin between production and reproduction, for the population is already at its most reproductive level.

Seed treatment, if the disease is of recent occurrence, should be temporarily beneficial, but should retard progress toward elimination of susceptible plants. If recurring mutation is responsible for holding susceptible plants in the population seed treatment, if it is completely effective, should be productive of equally beneficial results as long as it is used. Continued treatment should remain beneficial, but the population is subjected to changed environment in which the susceptible plants are no longer detrimentally effected. As a consequence, they are present in ever increasing proportion until, as a limit, the entire population is susceptible. If treatment were to cease, the results would be disastrous. If seed treatment is not completely effective, reproduction will gradually recede from its maximum in the first year of treatment, for the proportion of susceptible plants is increasing toward a new equilibrium and the results following cessation of treatment should be less disastrous than those under more efficient treatment.

If the third hypothesis holds, that is, if susceptibility is held in reciprocal balance in the population, seed treatment should be increasingly effective year by year until the population finally comes into a new equilibrium. On cessation of treatment reproduction should drop to a level below that maintained before treatment began and, in later years, should rise slowly to its original level.

GENERAL CONCLUSION

It may not be assumed that a hopeless picture is presented to the plant breeder. Only quantitative characters are considered, and, among these,

the margin between production and reproduction would seem to be relatively narrow only when improvement in grain or seed production is the characteristic sought for. Among self-fertilized plants hybrid combinations are constantly needed to permit selection for or by the slowly changing environment of a given community. Types with large seed or with poor vegetative vigor may at times come into the ascendency through human selection only. There may even be rare cases in which artificial selection is justified in the larger types to retain a heterozygous proportion above that at equilibrium.

Among random-fertilized plants there are also some benefits to be derived from selecting large seeded or poor pollen-producing types. When there exists selective progeny elimination as thinning, there could be utilized hybrid vigor which would result from the saving of the seed from the highly productive plants. First generation crosses between inbred strains show much economic promise, but, as has been pointed out by RICHEY and MAYER (1925), vigor of the inbred strains may be a poor criterion of yield in the hybrid.

SUMMARY

An effort is made to present in this paper as complete a discussion as possible of the genetic situation presented by a plant population when it has come into equilibrium, the processes by which equilibrium is reached, and the extent to which it may be modified by natural or by artificial selection. The distinction between production and reproduction is stressed and the point is made that natural selection is aimed at the latter, while, in artificial selection, the former is usually the goal. The reader is reminded that artificial selection may be either germinal or somatic. Germinal selection is the only type dealt with in this article.

It is assumed that the only two conditions capable of maintaining in a population allelomorphs of unequal reproductive capacity are (1) recurring mutation that is balanced against selective elimination, and (2) complete linkage between balanced detrimentals in order that the heterozygote may be favored by selection. Only the latter condition is studied. Hence the conclusions that are drawn should apply to the extent that this condition exists within the population.

A number of genetic formulas are presented. The more important ones for a self-fertilized population in which the generations do not overlap are given in the order of their derivation.

Formulas for finding F_{n+1} from F_n when R, S and T represent proportions

of AA, Aa, and aa and U, V, and W represent the corresponding reproductive capacities.

$$R' = \frac{UR + \frac{VS}{4}}{UR + VS + WT} \tag{1}$$

$$S' = \frac{\frac{VS}{2}}{UR + VS + WT} \tag{2}$$

$$T' = \frac{WT + \frac{VS}{4}}{UR + VS + WT} \tag{3}$$

Formulas for finding reproductive capacity of the heterozygote necessary to maintain equilibrium under inbreeding.

$$V = \frac{4UR}{2R - S} \tag{4}$$

$$V = \frac{4WT}{2T - S} \tag{5}$$

Formulas for finding proportions of R, S, and T at equilibrium under inbreeding when U, V, and W are known.

$$R = \frac{V(V-2W)}{V(V-2U) + V(V-2W) + 2(V-2U)(V-2W)} \tag{6}$$

$$S = \frac{2(V-2U)(V-2W)}{V(V-2U) + V(V-2W) + 2(V-2U)(V-2W)} \tag{7}$$

$$T = \frac{V(V-2U)}{V(V-2U) + V(V-2W) + 2(V-2U)(V-2W)} \tag{8}$$

Formulas for finding values of U and W in terms of V to maintain equilibrium under inbreeding when R, S, and T are known.

$$U = \frac{V(2R - S)}{4R} \tag{9}$$

$$W = \frac{V(2T - S)}{4T} \tag{10}$$

The more inportant formulas for a random-fertilized population follow: Formulas for finding F_{n+1} from F_n

$$R' = \frac{\left(UR + \frac{VS}{2}\right)^2}{(UR+VS+WT)^2} \tag{11}$$

$$S' = \frac{2\left(UR + \frac{VS}{2}\right)\left(WT + \frac{VS}{2}\right)}{(UR+VS+WT)^2} \tag{12}$$

$$T' = \frac{\left(WT + \frac{VS}{2}\right)^2}{(UR+VS+WT)^2} \tag{13}$$

Formula for finding reproductive capacity of the heterozygote necessary to maintain equilibrium under random-fertilization.

$$V = \frac{2R(US-2WT)}{S(2R-S)} \tag{14}$$

General formulas for determining conditions at equilibrium under random-fertilization.

$$T = \frac{V-U}{2V-(U+W)} \tag{15}$$

(This becomes identical with Wright's simplification of Fisher's formula for equilibrium if $\sqrt{T}$ be changed to Q, U to $1-L'$, V to 1, and W to $1-L''$.)

$$U = \frac{V(1-2\sqrt{T})+W\sqrt{T}}{1-\sqrt{T}} \tag{16}$$

$$V = \frac{U-\sqrt{T}(U+W)}{1-2\sqrt{T}} \tag{17}$$

$$W = \frac{\sqrt{T}(2V-U)-(V-U)}{\sqrt{T}} \tag{18}$$

$$U = 1$$

$$\sqrt{T} = \frac{1}{2+H} \tag{19}$$

$$W = -1\frac{1}{L} \tag{20}$$

$$V = 1 + \frac{1}{HL} \tag{21}$$

To the extent that valid deductions may be drawn from the results of these mathematical processes, the following conclusions are pertinent:

(1) Barring mutation, a population under a constant environment approaches an equilibrium predetermined by the reproductive capacities of the genotypes present.

(2) At equilibrium the population may contain (a) single homozygotes, (b) equally reproductive allelomorphs, (c) the products of ever mutating factors, and (d) factors reciprocally balanced by complete linkage so that selection favors the heterozygote. Before random-bred forms may retain factors in reciprocal balance, the heterozygote must be more reproductive than either homozygote. Before inbred forms may retain factors in reciprocal balance the heterozygote must be more than twice as reproductive as either homozygote.

(3) A random-bred population at equilibrium contains the different genotype in such proportion that reproduction for the population is at its maximum.

(4) An inbred population at equilibrium need not contain the different genotypes in such proportion that reproduction for the population is at its maximum.

(5) An altered environment that changes the reproductive capacity of a reciprocally balanced homozygote induces a new equilibrium at a new reproductive level. A change relatively detrimental to a homozygote of such a balanced set induces the population to seek a new equilibrium on a lower reproductive level with the affected homozygote present in reduced proportion. Conversely a change relatively beneficial to a homozygote of such a balanced set induces the population to seek a new equilibrium on a higher reproductive level with the affected homozygote present in increased proportion.

On account of this regularity in behavior it may be concluded further that:

(6) When a plant or animal comes into such an environment that factors once detrimental are no longer so, bizarre forms may gain the ascendency and wipe out characteristics once essential to the existence of the species.

(7) Limited selection for sparse inflorescence should increase grain or fruit yield on a poor soil.

(8) In a random-bred population selection for sparse pollen should increase yield of grain or fruit.

(9) Selection for increase in seed size should usually cause increase in yield of a grain plant.

(10) Within a single random-bred population there seems to be little cause for remixing inbred strains in an effort to synthesize a permanent highly reproductive variety that lacks the detrimentals of the parent population.

(11) The more the detriment of a factor in reciprocal balance, and the higher the equilibrium in which it is held, the greater should be the hybrid vigor of its heterozygote.

(12) Finally, the general conclusion must be: Barring mutation in random-bred forms, man is enabled to effect and maintain germinal improvement by selection to the extent that he utilizes the margin of variation between reproductive ability and productive ability within the environment in which the form is grown.

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